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(54) Title: GENE THERAPY FOR EFFECTOR CELL REGULATION

(57) Abstract

The present invention provides a nucleic acid-based therapeutic composition to treat an animal with disease by controlling the activity of effector cells, including T cells, macrophages, monocytes and/or natural killer cells, in the animal. Therapeutic compositions of the present invention include superantigen-encoding nucleic acid molecules, either in the presence or absence of a cytokine-encoding nucleic acid molecule and/or chemokine-encoding nucleic acid molecules, depending upon the disease being treated. The present invention also relates to an adjuvant for use with nucleic acid-based vaccines. Adjuvant compositions of the present invention include an immunogen combined with superantigen-encoding nucleic acid molecules, either in the presence or absence of a cytokine-encoding nucleic acid molecule and/or chemokine-encoding nucleic acid molecules.

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GENE THERAPY FOR EFFECTOR CELL REGULATION

FIELD OF THE INVENTION

The present invention relates to a product and process for regulating T cell activity by providing a superantigen gene, in the presence or absence of a cytokine and/or chemokine gene. The present invention also relates to a product and process for regulating T cell activity by providing a peptide and a superantigen gene, in the presence or absence of a cytokine and/or chemokine gene. In particular, the present invention relates to a product and process for controlling tumor development, immune responses to infectious diseases and diseases caused by immunological disorders.

BACKGROUND OF THE INVENTION

Two major causes of disease include infectious agents and malfunctions of normal biological functions of an animal. Examples of infectious agents include viruses, bacteria, parasites, yeast and other fungi. Examples of abnormal biological function include uncontrolled cell growth, abnormal immune responses and abnormal inflammatory responses. Traditional reagents used attempt to protect an animal from disease include reagents that destroy infectious agents or cells involved in deregulated biological functions. Such reagents, however, can result in unwanted side effects. For example, anti-viral drugs that disrupt the replication of viral DNA also often disrupt DNA replication in normal cells in the treated patient. Other treatments with chemotherapeutic reagents to

destroy cancer cells typically leads to side effects, such as bleeding, vomiting, diarrhea, ulcers, hair loss and increased susceptibility to secondary cancers and infections.

An alternative method of disease treatment includes modulating the immune system of a patient to assist the patient's natural defense mechanisms. Traditional reagents and methods used to attempt to regulate an immune response in a patient also result in unwanted side effects and have limited effectiveness. For example, immunosuppressive reagents (e.g., cyclosporin A, azathioprine, and prednisone) used to treat patients with autoimmune disease also suppress the patient's entire immune response, thereby increasing the risk of infection. In addition, immunopharmacological reagents used to treat cancer (e.g., interleukins) are short-lived in the circulation of a patient and are ineffective except in large doses. Due to the medical importance of immune regulation and the inadequacies of existing immunopharmacological reagents, reagents and methods to regulate specific parts of the immune system have been the subject of study for many years.

Stimulation or suppression of the immune response in a patient can be an effective treatment for a wide variety of medical disorders. T lymphocytes (T cells) are one of a variety of distinct cell types involved in an immune response. The activity of T cells is regulated by antigen, presented to a T cell in the context of a major

histocompatibility complex (MHC) molecule. The T cell receptor (TCR) then binds to the MHC:antigen complex. Once antigen is complexed to MHC, the MHC:antigen complex is bound by a specific TCR on a T cell, thereby altering the activity of that T cell.

The use of certain staphylococcal enterotoxin proteins that are capable of complexing with MHC molecules to influence T cell function has been suggested by various investigators, including, for example, White et al., *Cell* 56:27-35, 1989; Rellahan et al. *J. Expt. Med.* 172:1091-1100, 1990; Micusan et al., *Immunology* 5:3-11, 1993; Hermann et al., *Immunology* 5:33-39, 1993; Bhardwaj et al., *J. Expt. Med.* 178:633-642, 1993; and Kalland et al., *Med. Oncol. & Tumor Pharmacother.*, 10:37-47, 1993. In particular, various investigators have suggested that *Staphylococcal enterotoxin* proteins are useful for treating tumors, including Newell et al., *Proc. Natl. Acad. Sci. USA* 88:1074-1078, 1991; Kalland et al., PCT Application No. WO 91/04053, published April 4, 1991; Dohlstein et al., *Proc. Natl. Acad. Sci. USA* 88:9287-9291, 1991; Hedlund et al., *Cancer Immunol. Immunother.* 36:89-93, 1993; Lando et al., *Cancer Immunol. Immunother.* 36:223-228, 1993; Lukacs et al., *J. Exp. Med.* 178:343-348, 1993; Ochi et al., *J. Immunol.* 151:3180-3186, 1993; and Terman et al., PCT Application No. WO 93/24136, published December 9, 1993. These investigators, however, have only disclosed the use of bacterial enterotoxin proteins themselves. The use of

bacterial enterotoxin protein has the major disadvantage of being toxic to the recipient of the protein.

Thus, there is a need for a product and process that allows for the treatment of disease using bacterial enterotoxins in a non-toxic manner.

SUMMARY

Traditional pharmaceutical reagents used to treat cancer, infectious diseases and diseases caused by immunological disorders often have harmful side effects. In addition, such reagents can be unpredictable (e.g., treatment of cancer, vaccination against infectious agents). For example, chemotherapy and radiotherapy often cause extensive normal tissue damage during the process of treating cancerous tissue. In addition, vaccine treatments for the prevention or cure of infectious diseases are often ineffective because adjuvants useful in vaccine therapy are toxic to an animal.

The present invention is particularly advantageous in that it provides an effective therapeutic composition that enables the safe treatment of an animal with a reagent that is a potentially toxic an immunogenic protein. Upon delivery, expression of acid molecules contained in the therapeutic composition result in localized production of an effective but non-toxic amount of encoded proteins that may be toxic at concentrations that would be required if the encoded proteins were administered directly. The therapeutic compositions of the present invention can

provide long term expression of the encoded proteins at a site in an animal. Such long term expression allows for the maintenance of an effective, but non-toxic, dose of the encoded protein to treat a disease and limits the frequency of administration of the therapeutic composition needed to treat an animal. In addition, because of the lack of toxicity, therapeutic compositions of the present invention can be used in repeated treatments.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 illustrates the expression of superantigen-encoding DNA plasmids in mammalian cells.

Fig. 2 illustrates the proliferative response of canine PBMC's to canine melanoma cells transfected with a superantigen-encoding DNA plasmids.

Figs. 3A and 3B illustrate the release of superantigen protein by CHO cells transfected with superantigen-encoding DNA plasmids.

Fig. 4 illustrates the proliferative response of the Vβ3+ T cell clone AD10 to melanoma cells transfected with superantigen-encoding DNA plasmid.

Fig. 5 illustrates the release of canine GM-CSF by CHO cells transfected with GM-CSF-encoding DNA plasmid.

Figs. 6A and 6B illustrate the vaccination of mice with autologous tumor cells transfected with superantigen-encoding DNA plasmid.

Fig. 7 illustrates the effect of tumor target transfection on cytotoxic T cell lysis.

Fig. 8 illustrates the response of Vβ3+ T cells to intramuscular injection of a superantigen-encoding DNA plasmid.

Fig. 9 illustrates the antibody response resulting from the co-administration of DNA encoding an adjuvant and DNA encoding ovalbumin.

Fig. 10 illustrates that the co-administration of DNA encoding an adjuvant and DNA encoding ovalbumin increase interferon-gamma release from T cells restimulated *in vitro* by the ovalbumin protein.

Fig. 11 illustrates that the co-administration of DNA encoding an adjuvant and DNA encoding ovalbumin increase T cell proliferative responses to ovalbumin.

Fig. 12 illustrates that the co-administration of DNA encoding an adjuvant and DNA encoding ovalbumin increases CTL responses to ovalbumin.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a novel product and process for controlling effector cell activity. The present invention also relates to a novel adjuvant useful for enhancing an immune response. It is now known for the first time that a composition containing nucleic acid molecules encoding a superantigen, rather than superantigen proteins, is an effective therapeutic reagent for treating disease and is an effective adjuvant for enhancing an immune response. As used herein, a disease refers to any biological abnormality that is not beneficial to a subject.

The present inventors have also discovered that administration of a combination of nucleic acid molecules encoding: (1) a superantigen; (2) a superantigen and a cytokine; or (3) a superantigen and a chemokine, can act synergistically to effectively treat cancer and infectious disease. The present invention includes therapeutic compositions comprising: (a) an isolated nucleic acid molecule encoding a superantigen; or (b) an isolated nucleic acid molecule encoding a superantigen in combination with an isolated nucleic acid molecule encoding a cytokine and/or an isolated nucleic acid molecule encoding a chemokine. Administration of a therapeutic composition of the present invention to an animal results in the production of superantigen, cytokine or chemokine proteins, referred to herein as "encoded proteins." Each of the components of a therapeutic composition of the present invention is described in detail below, followed by a description of the methods by which the therapeutic composition is used and delivered.

One embodiment of the present invention includes a method for increasing effector cell immunity in an animal, the method comprising administering to an animal an effective amount of a therapeutic composition comprising: (a) an isolated nucleic acid molecule encoding a superantigen; or (b) an isolated nucleic acid molecule encoding a superantigen in combination with an isolated nucleic acid molecule encoding a cytokine and/or an isolated nucleic acid molecule encoding chemokine.

According to the present embodiment, the nucleic acid molecules are operatively linked to one or more transcription control sequences and the therapeutic composition is targeted to a site in the animal that contains an abnormal cell. According to the present invention, an effector cell, includes a helper T cell, a cytotoxic T cell, a macrophage, a monocyte and/or a natural killer cell. For example, the method of the present invention can be performed to increase the number of effector cells in an animal that are capable of killing or releasing cytokines or chemokines when presented with antigens derived from an abnormal cell or a pathogen. An effective amount of a therapeutic composition of the present invention comprises an amount capable of treating a disease as described herein. Alternatively, a method of the present invention can be performed to decrease the number of T cells found in a T cell subset that is preferentially stimulated and expanded by an autoantigen.

As used herein, effector cell immunity refers to increasing the number and/or the activity of effector cells in the area of the abnormal cell. In particular, T cell activity refers to increasing the number and/or the activity of T cells in the area of the abnormal cell. Also, as used herein, an abnormal cell refers to a cell displaying abnormal biological function, such as abnormal growth, development or death. Abnormal cells of the present invention, preferably includes cancer cells, cells infected with an infectious agent (i.e., a pathogen) and non-

cancerous cells having abnormal proliferative growth (e.g., sarcoidosis, granulomatous disease or papillomas) and with cancer cells and infected cells. Another embodiment of the present invention is a method to treat an animal with cancer, the method comprising administering to an animal an effective amount of a therapeutic composition comprising: (a) a nucleic acid molecule encoding a superantigen; or (b) a nucleic acid molecule encoding a superantigen in combination with an isolated nucleic acid molecule encoding a cytokine and/or a nucleic acid molecule encoding a chemokine. According to the present embodiment, the nucleic acid molecules are operatively linked to one or more transcription control sequences and the therapeutic composition is targeted to the site of a cancer.

One embodiment of a therapeutic composition of the present invention comprises an isolated nucleic acid molecule encoding a superantigen (also referred to herein as a "superantigen-encoding" nucleic acid molecule). Another embodiment of a therapeutic composition of the present invention comprises an isolated nucleic acid molecule encoding a superantigen, combined with an isolated nucleic acid molecule encoding a cytokine (also referred to herein as a "cytokine-encoding" nucleic acid molecule) and/or a nucleic acid molecule encoding a chemokine (also referred to as a "chemokine-encoding" nucleic acid molecule). According to these embodiments, the nucleic acid molecules are operatively linked to one or more transcription control sequences. It is to be noted that

the term "a" or "an" entity refers to one or more of that entity; for example, a compound refers to one or more compounds. As such, the terms "a" (or "an"), "one or more" and "at least one" can be used interchangeably herein. According to the present invention, an isolated, or biologically pure, nucleic acid molecule, is a nucleic acid molecule that has been removed from its natural milieu. As such, "isolated" and "biologically pure" do not necessarily reflect the extent to which the nucleic acid molecule has been purified. An isolated nucleic acid molecule can include DNA, RNA, or derivatives of either DNA or RNA. An isolated superantigen or cytokine nucleic acid molecule can be obtained from its natural source, either as an entire (i.e., complete) gene or a portion thereof capable of encoding a superantigen protein capable of binding to an MHC molecule or a cytokine protein capable of binding to a complementary cytokine receptor. A nucleic acid molecule can also be produced using recombinant DNA technology (e.g., polymerase chain reaction (PCR) amplification, cloning) or chemical synthesis. Nucleic acid molecules include natural nucleic acid molecules and homologues thereof, including, but not limited to, natural allelic variants and modified nucleic acid molecules in which nucleotides have been inserted, deleted, substituted, and/or inverted in such a manner that such modifications do not substantially interfere with the nucleic acid molecule's ability to encode a functional superantigen or a functional cytokine of the present invention.

A nucleic acid molecule homologue can be produced using a number of methods known to those skilled in the art (see, for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press, 1989). For example, nucleic acid molecules can be modified using a variety of techniques including, but not limited to, classic mutagenesis techniques and recombinant DNA techniques, such as site-directed mutagenesis, chemical treatment of a nucleic acid molecule to induce mutations, restriction enzyme cleavage of a nucleic acid fragment, ligation of nucleic acid fragments, polymerase chain reaction (PCR) amplification and/or mutagenesis of selected regions of a nucleic acid sequence, synthesis of oligonucleotide mixtures and ligation of mixture groups to "build" a mixture of nucleic acid molecules and combinations thereof. Nucleic acid molecule homologues can be selected from a mixture of modified nucleic acids by screening for the function of the protein encoded by the nucleic acid (e.g., superantigen, cytokine or chemokine activity, as appropriate). Techniques to screen for superantigen, cytokine or chemokine activity are known to those of skill in the art.

Although the phrase "nucleic acid molecule" primarily refers to the physical nucleic acid molecule and the phrase "nucleic acid sequence" primarily refers to the sequence of nucleotides on the nucleic acid molecule, the two phrases can be used interchangeably, especially with respect to a nucleic acid molecule, or a nucleic acid sequence, being

capable of encoding a superantigen, a cytokine or a chemokine protein. In addition, the phrase "recombinant molecule" primarily refers to a nucleic acid molecule operatively linked to a transcription control sequence, but can be used interchangeably with the phrase "nucleic acid molecule" which is administered to an animal. As heretofore disclosed, superantigen or cytokine proteins of the present invention include, but are not limited to, proteins having full-length superantigen, cytokine or chemokine coding regions, proteins having partial superantigen regions capable of binding to an MHC molecule, cytokine coding regions capable of binding to a complementary cytokine receptor, chemokine coding regions capable of binding to a complementary chemokine receptor, fusion proteins and chimeric proteins comprising combinations of different superantigens, cytokines and/or chemokines.

One embodiment of the present invention is an isolated superantigen-encoding nucleic acid molecule that encodes at least a portion of a full-length superantigen, or a homologue of a superantigen. As used herein, "at least a portion of a superantigen" refers to a portion of a superantigen protein capable of binding to an MHC molecule in such a manner that a TCR can bind to the resulting superantigen:MHC complex. Preferably, a superantigen nucleic acid molecule of the present invention encodes an entire coding region of a superantigen, and more preferably the coding region absent a leader sequence. Production of a truncated superantigen protein lacking a bacterial leader

sequence is preferred to enhance secretion of the superantigen from a cell. As used herein, a homologue of a superantigen is a protein having an amino acid sequence that is sufficiently similar to a natural superantigen amino acid sequence that a nucleic acid sequence encoding the homologue encodes a protein capable of binding to an MHC molecule.

In accordance with the present invention, a superantigen comprises a family of T cell regulatory proteins that are capable of binding both to an MHC molecule. A superantigen binds to the extracellular portion of an MHC molecule to form and MHC:superantigen complex. The activity of a T cell can be modified when a TCR binds to an MHC:superantigen complex. Under certain circumstances, an MHC:superantigen complex can have a mitogenic role (i.e., the ability to stimulate the proliferation of T cells) or a suppressive role (i.e., deletion of T cell subsets). The ability of an MHC:superantigen complex to have a stimulatory or suppressive role can depend upon factors, such as the concentration and environment (i.e., tissue location and/or the presence of cytokines).

The mitogenic role of a superantigen is distinct from other known mitogens (e.g., lectins derived from plants) in that superantigens are capable of stimulating the proliferation of particular subsets of T cells having TCR's that specifically bind to the superantigen. For example, a superantigen, when added to a mixed lymphocyte

population, is able to stimulate the proliferation of a select population of T cells from the mixed population of cells. Examples of T cell subsets stimulated by superantigens complexed with MHC molecules include T cells expressing a TCR comprising mouse $V_\beta 1$, $V_\beta 3$, $V_\beta 7$, $V_\beta 8.1$, $V_\beta 8.2$, $V_\beta 8.3$, $V_\beta 10$, $V_\beta 11$, $V_\beta 17$, $V_\beta 15$ or $V_\beta 16$ chains, and T cells expressing a TCR comprising human $V_\beta 1.1$, $V_\beta 2$, $V_\beta 3$, $V_\beta 5$, $V_\beta 6$, $V_\beta 7.3$, $V_\beta 7.4$, $V_\beta 9.1$, $V_\beta 12$, $V_\beta 14$, $V_\beta 15$, $V_\beta 17$ or $V_\beta 20$ chains.

A superantigen-encoding nucleic acid molecule of the present invention preferably encodes superantigens that includes, but is not limited to, staphylococcal enterotoxins, retroviral antigens, streptococcal antigens, mycoplasma antigens, mycobacterium antigens, viral antigens (e.g., a superantigen from mouse mammary tumor virus, rabies virus or herpes virus) and endoparasitic antigens (e.g., protozoan or helminth antigens), more preferably staphylococcal enterotoxins, and even more preferably *Staphylococcal enterotoxin* A (SEA), *Staphylococcal enterotoxin* B (SEB), *Staphylococcal enterotoxin* C_1 (SEC_1), *Staphylococcal enterotoxin* C_2 (SEC_2), *Staphylococcal enterotoxin* C_3 (SEC_3), *Staphylococcal enterotoxin* D (SED), *Staphylococcal enterotoxin* E (SEE) and Toxic Shock Syndrome Toxin (TSST).

A preferred nucleic acid molecule encoding a *Staphylococcal enterotoxin* of the present invention comprises a nucleic acid sequence represented by SEQ ID NO:1 (representing a full-length SEB gene), SEQ ID NO:3

(representing a full-length SEA gene) or SEQ ID NO:6 (representing a full-length TSST gene). A preferred *Staphylococcal enterotoxin* protein of the present invention comprises an amino acid sequence represented by SEQ ID NO:2 (representing a full-length SEB protein), SEQ ID NO:4 (representing a full-length SEA protein) or SEQ ID NO:7 (representing a full-length TSST protein).

In a preferred embodiment, a nucleic acid molecule of the present invention encoding a superantigen comprises a nucleic acid sequence spanning base pair 46 to at least base pair 768 of SEQ ID NO:1, a nucleic acid sequence spanning base pair 46 to about base pair 751 of SEQ ID NO:3 or SEQ ID NO:6.

Another embodiment of the present invention includes a cytokine-encoding nucleic acid molecule that encodes a full-length cytokine or a homologue of the cytokine protein. As used herein, a homologue of a cytokine is a protein having an amino acid sequence that is sufficiently similar to a natural cytokine amino acid sequence so as to have cytokine activity. In accordance with the present invention, a cytokine includes a protein that is capable of affecting the biological function of another cell. A biological function affected by a cytokine can include, but is not limited to, cell growth, cell differentiation or cell death. Preferably, a cytokine of the present invention is capable of binding to a specific receptor on the surface of a cell, thereby affecting the biological function of a cell.

A cytokine-encoding nucleic acid molecule of the present invention encodes a cytokine that is capable of affecting the biological function of a cell, including, but not limited to, a lymphocyte, a muscle cell, a hematopoietic precursor cell, a mast cell, a natural killer cell, a macrophage, a monocyte, an epithelial cell, an endothelial cell, a dendritic cell, a mesenchymal cell, a Langerhans cell, cells found in granulomas and tumor cells of any cellular origin, and more preferably a mesenchymal cell, an epithelial cell, an endothelial cell, a muscle cell, a macrophage, a monocyte, a T cell and a dendritic cell.

A preferred cytokine nucleic acid molecule of the present invention encodes a hematopoietic growth factor, an interleukin, an interferon, an immunoglobulin superfamily molecule, a tumor necrosis factor family molecule and/or a chemokine (i.e., a protein that regulates the migration and activation of cells, particularly phagocytic cells). A more preferred cytokine nucleic acid molecule of the present invention encodes a granulocyte macrophage colony stimulating factor (GM-CSF), tumor necrosis factor α (TNF-α), macrophage colony stimulating factor (M-CSF), interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-12 (IL-12), interleukin-15 (IL-15) and/or IGIF. An even more preferred cytokine nucleic acid molecule of the present invention encodes GM-CSF, IL-2, IL-12, IGIF and/or TNF-α, with GM-CSF being even more preferred.

As will be apparent to one of skill in the art, the present invention is intended to apply to cytokines derived from all types of animals. A preferred animal from which to derive cytokines includes a mouse, a human, a cat and a dog. A more preferred animal from which to derive cytokines includes a cat, a dog and a human. An even more preferred animal from which to derive cytokines is a human.

According to the present invention, a cytokine-encoding nucleic acid molecule of the present invention is derived from the same species of animal as the animal to be treated. For example, a cytokine-encoding nucleic acid molecule derived from a canine (i.e., dog) nucleic acid molecule is used to treat a disease in a canine. Thus, a preferred cytokine-encoding nucleic acid molecule of the present invention comprises a nucleic acid molecule encoding human GM-CSF, as described in the art. A human GM-CSF-encoding nucleic acid molecule of the present invention can be produced using methods standard PCR amplification methods with primers designed from the human GM-CSF nucleic acid sequence disclosed in Nash (*Blood* 78:930, 1991). Such PCR products can be cloned into a PCR_3 expression vector using the methods generally described in Example 1.

Another embodiment of the present invention includes a chemokine-encoding nucleic acid molecule that encodes a full-length chemokine or a homologue of the chemokine protein. As used herein, a homologue of a chemokine is a protein having an amino acid sequence that is sufficiently

similar to a natural chemokine amino acid sequence so as to have chemokine activity. In accordance with the present invention, a chemokine includes a protein that is capable of attracting cells involved in an immune response (immunologic cells), including phagocytic cells. For example, immunologic cells are recruited from the blood to a site at which the chemokine is located (e.g., a site of infection). Preferably, a chemokine of the present invention is capable of binding to a specific receptor on the surface of a cell, thereby attracting the cell to a specific location.

A chemokine-encoding nucleic acid molecule of the present invention encodes a chemokine that is capable of attracting a cell to a site, including, but not limited to, a dendritic cell, a neutrophil, a macrophage, a T lymphocyte and Langerhans cells, and more preferably a dendritic cell, a macrophage and a T lymphocyte.

A preferred chemokine-encoding nucleic acid molecule of the present invention encodes an α-chemokine or a β-chemokine. A more preferred chemokine-encoding nucleic acid molecule of the present invention encodes a C5a, interleukin-8 (IL-8), monocyte chemotactic protein 1α (MIP1α), monocyte chemotactic protein 1β (MIP1β), monocyte chemoattractant protein 1 (MCP-1), monocyte chemoattractant protein 3 (MCP-3), platelet activating factor (PAFR), N-Formyl-methionyl-leucyl-[^{3H}]phenylalanine (FMLPR), leukotriene B_4 (LTB_4R), gastrin releasing peptide (GRP), RANTES, eotaxin, lymphotactin, IP10, I-309, ENA78, GCP-2,

NAP-2 and/or MGSA/gro. An even more preferred chemokine-encoding nucleic acid molecule of the present invention encodes IL-8, MIP1α, MIP1β, MCP-1, MCP-3, RANTES and/or NAP-2, with IL-8, Rantes, MIP1α and/or MIP1β being even more preferred.

As will be apparent to one of skill in the art, the present invention is intended to apply to chemokines derived from all types of animals. Preferred animals from which to derive chemokines includes mice, humans, dogs, cats, cattle and horses. More preferred animals from which to derive chemokines includes dogs, cats, humans and cattle. Even more preferred animals from which to derive chemokines are humans.

According to the present invention, a chemokine-encoding nucleic acid molecule of the present invention is derived from the same species of animal as the animal to be treated. For example, a chemokine-encoding nucleic acid molecule derived from a canine (i.e., dog) nucleic acid molecule is used to treat a disease in a canine. Thus, a preferred chemokine-encoding nucleic acid molecule of the present invention comprises a nucleic acid molecule encoding a dog, cat, human, bovine and/or equine chemokine. Preferred nucleic acid molecules of the present invention encode IL-8, Rantes, MIP1α and/or MIP1β, as described in the art. For example, a human MIP1α-encoding nucleic acid molecule of the present invention can be produced using standard PCR amplification methods with primers designed from the human MIP1α-encoding nucleic acid sequence

disclosed in the art. Such PCR products can be cloned into a PCR_3 expression vector using the methods generally described in Example 1.

The present invention includes a nucleic acid molecule of the present invention operatively linked to one or more transcription control sequences to form a recombinant molecule. The phrase "operatively linked" refers to linking a nucleic acid molecule to a transcription control sequence in a manner such that the molecule is able to be expressed when transfected (i.e., transformed, transduced or transfected) into a host cell. Transcription control sequences are sequences which control the initiation, elongation, and termination of transcription. Particularly important transcription control sequences are those which control transcription initiation, such as promoter, enhancer, operator and repressor sequences. Suitable transcription control sequences include any transcription control sequence that can function in at least one of the recombinant cells of the present invention. A variety of such transcription control sequences are known to those skilled in the art. Preferred transcription control sequences include those which function in animal, bacteria, helminth, insect cells, and preferably in animal cells. More preferred transcription control sequences include, but are not limited to, simian virus 40 (SV-40), β-actin, retroviral long terminal repeat (LTR), Rous sarcoma virus (RSV), cytomegalovirus (CMV), *tac*, *lac*, *trp*, *trc*, oxy-pro, omp/lpp, rrnB, bacteriophage lambda (λ) (such as λp_L and λp_R

and fusions that include such promoters), bacteriophage T7, T7*lac*, bacteriophage T3, bacteriophage SP6, bacteriophage SP01, metallothionein, alpha mating factor, *Pichia* alcohol oxidase, alphavirus subgenomic promoters (such as Sindbis virus subgenomic promoters), baculovirus, *Heliothis zea* insect virus, vaccinia virus and other poxviruses, herpesvirus, and adenovirus transcription control sequences, as well as other sequences capable of controlling gene expression in eukaryotic cells. Additional suitable transcription control sequences include tissue-specific promoters and enhancers (e.g., tumor cell-specific enhancers and promoters), and inducible promoters (e.g., tetracycline). Transcription control sequences of the present invention can also include naturally occurring transcription control sequences naturally associated with a gene encoding a superantigen, a cytokine or a chemokine of the present invention.

Recombinant molecules of the present invention, which can be either DNA or RNA, can also contain additional regulatory sequences, such as translation regulatory sequences, origins of replication, and other regulatory sequences that are compatible with the recombinant cell. In one embodiment, a recombinant molecule of the present invention also contains secretory signals (i.e., signal segment nucleic acid sequences) to enable an expressed superantigen, cytokine or a chemokine protein to be secreted from the cell that produces the protein. Suitable signal segments include: (1) a bacterial signal segment, in

particular a superantigen signal segment; (2) a cytokine signal segment; (3) a chemokine signal segment; (4) or any heterologous signal segment capable of directing the secretion of a superantigen, cytokine and/or chemokine protein of the present invention. Preferred signal segments include, but are not limited to, signal segments associated with SEB, SEA, TSST, GM-CSF, M-CSF, TNFα, IL-1, IL-2, IL-4, IL-6, IL-12, IL-15, C5a, IGIF, IL-8, MIP1α, MIP1β, MCP-1, MCP-3, PAFR, FMLPR, LTB_4R, GRP, RANTES, eotaxin, lymphotactin, IP10, I-309, ENA78, GCP-2, NAP-2 and/or MGSA/gro protein.

Preferred recombinant molecules of the present invention include a recombinant molecule containing a nucleic acid molecule encoding a superantigen, a recombinant molecule containing a nucleic acid molecule encoding a cytokine, a recombinant molecule containing a nucleic acid molecule encoding a chemokine, a recombinant molecule containing a nucleic acid molecule encoding a superantigen and a nucleic acid molecule encoding a cytokine to form a chimeric recombinant molecule, or a recombinant molecule containing a nucleic acid molecule encoding a superantigen and a nucleic acid molecule encoding a chemokine to form a chimeric recombinant molecule. The nucleic acid molecules contained in such recombinant chimeric molecules are operatively linked to one or more transcription control sequences, in which each nucleic acid molecule contained in a chimeric recombinant molecule can be expressed using the same or different

regulatory control sequences. Preferred recombinant molecules of the present invention comprise a nucleic acid sequence represented by SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, or combinations thereof. Particularly preferred recombinant molecules include PCR_3-SEB, PCR_3-SEA, PCR_3-SEB.S, PCR_3-SEA.S, PCR_3-TSST and PCR_3-GM_3, the production of which is disclosed herein. Other preferred nucleic acid sequences include Rantes nucleic acid sequence (SEQ ID. NO:13), MIP1α nucleic acid sequence (see Davatelis et al., *J. Exp. Med.* 167:1939-1944, 1988) and MIP1β nucleic acid sequence (see Sherry et al., *J. Exp. Med.* 168:2251-2259, 1988).

According to the present invention, a recombinant molecule can be dicistronic. A cistron refers to a unit of DNA that is capable of encoding an amino acid sequence having a naturally-occurring biological function. A dicistronic plasmid refers to a plasmid containing 2 cistrons. Preferably, a dicistronic recombinant molecule of the present invention comprises an internal ribosome entry site (IRES) element to which eukaryotic ribosomes can bind (see, for example, Jang et al., *J. Virol.* 62:2636-2643, 1988; Pelletier et al. *Nature* 334:320-325, 1988; Jackson, *Nature* 353:14-15, 1991; Macejek et al., *Nature* 353:90-94, 1991; Oh et al., *Genes & Develop.* 6:1643-1653, 1992; Molla et al., *Nature* 356:255-257, 1992; and Kozak, *Crit. Rev. Biochem. Molec. Biol.* 27(4,5):385-402, 1992).

In one embodiment, a dicistronic recombinant molecule of the present invention comprises a eukaryotic promoter, operatively linked to a superantigen-encoding nucleic acid

molecule of the present invention and a cytokine-encoding nucleic acid molecule of the present invention separated by an IRES nucleic acid sequence, or a superantigen-encoding nucleic acid molecule of the present invention and chemokine-encoding nucleic acid molecule of the present invention separated by an IRES nucleic acid sequence.

In another embodiment, a dicistronic recombinant molecule of the present invention comprises a eukaryotic promoter, operatively linked to a first superantigen-encoding nucleic acid molecule of the present invention and a second superantigen-encoding nucleic acid molecule of the present invention separated by an IRES nucleic acid sequence. Preferably, a first superantigen-encoding nucleic acid molecule encodes a different superantigen than a second superantigen-encoding nucleic acid molecule.

One or more recombinant molecules of the present invention can be used to produce an encoded product (i.e., a superantigen protein, a cytokine and a chemokine protein) of the present invention. In one embodiment, an encoded product of the present invention is produced by expressing a nucleic acid molecule of the present invention in a cell under conditions effective to produce the protein. A preferred method to produce an encoded protein is by transforming (i.e., introducing a recombinant molecule into a cell in such a manner that the recombinant molecule is expressed by the cell) a host cell with one or more recombinant molecules of the present invention to form a recombinant cell. Suitable host cells to transform include

any cell into which a recombinant molecule can be introduced. Host cells can be either untransformed cells or cells that are already transformed with at least one nucleic acid molecule. Host cells of the present invention can be any cell capable of producing a superantigen, a cytokine and/or a chemokine of the present invention, including bacterial, fungal, animal parasite, insect and animal cells. A preferred host cell includes a mammalian and a bird cell. A more preferred host cell includes mammalian lymphocytes, muscle cells, hematopoietic precursor cells, mast cells, natural killer cells, macrophages, monocytes, epithelial cells, endothelial cells, dendritic cells, mesenchymal cells, Langerhans cells, cells found in granulomas and tumor cells of any cellular origin. An even more preferred host cell of the present invention includes mammalian mesenchymal cells, epithelial cells, endothelial cells, macrophages, monocytes, muscle cells, T cells and dendritic cells.

According to the present invention, a recombinant molecule can be introduced into a host cell *in vivo* (i.e., in an animal) or *in vitro* (i.e., outside of an animal, such as in tissue culture). Introduction of a nucleic acid molecule into a host cell can be accomplished by any method by which a nucleic acid molecule can be inserted into the cell. Transformation techniques include, but are not limited to, transfection, electroporation, microinjection, lipofection, adsorption, and protoplast fusion. Preferred methods to introduce a recombinant molecule into host cells

in vivo include lipofection and adsorption (discussed in detail below).

A recombinant cell of the present invention comprises a cell into which a nucleic acid molecule that encodes a superantigen, a cytokine and/or a chemokine has been introduced. In one embodiment, a recombinant cell of the present invention is transformed with a nucleic acid molecule that includes at least a portion of PCR_3-SEB, PCR_3-SEA, PCR_3-SEB.S, PCR_3-SEA.S, PCR_3-TSST, or combinations thereof. Particularly preferred recombinant cells include cells transformed with PCR_3-SEB, PCR_3-SEA, PCR_3-SEB.S, PCR_3-SEA.S or PCR_3-TSST, with PCR_3-SEB.S, PCR_3-SEA.S or PCR_3-TSST being even more preferred.

In another embodiment, a recombinant cell of the present invention is transformed with a nucleic acid molecule that includes at least a portion of PCR_3-SEB, PCR_3-SEA, PCR_3-SEB.S, PCR_3-SEA.S, PCR_3-TSST or combinations thereof, and PCR_3-GM_3. Particularly preferred stimulatory recombinant cells include cells transformed with PCR_3-SEA and PCR_3-GM_3, PCR_3-SEA.S and PCR_3-GM_3, PCR_3-SEB and PCR_3-GM_3, PCR_3-SEB.S and PCR_3-GM_3, or PCR_3-TSST and PCR_3-GM_3. Even more preferred stimulatory recombinant cells include cells transformed with PCR_3-SEB.S and PCR_3-GM_3, or PCR_3-SEA.S and PCR_3-GM_3, and PCR_3-TSST and PCR_3-GM_3.

Recombinant DNA technologies can be used to improve expression of transformed nucleic acid molecules by manipulating, for example, the number of copies of the nucleic acid molecules within a host cell, the efficiency

with which those nucleic acid molecules are transcribed, the efficiency with which the resultant transcripts are translated, and the efficiency of post-translational modifications. Recombinant techniques useful for increasing the expression of nucleic acid molecules of the present invention include, but are not limited to, operatively linking nucleic acid molecules to high-copy number plasmids, integration of the nucleic acid molecules into one or more host cell chromosomes, addition of vector stability sequences to plasmids, substitutions or modifications of transcription control signals (e.g., promoters, operators, enhancers), substitutions or modifications of translational control signals (e.g., ribosome binding sites, Shine-Dalgarno sequences), modification of nucleic acid molecules of the present invention to correspond to the codon usage of the host cell, and deletion of sequences that destabilize transcripts. The activity of an expressed recombinant protein of the present invention may be improved by fragmenting, modifying, or derivatizing nucleic acid molecules encoding such a protein.

Further embodiments of compositions of the present invention can also include a compound capable of inhibiting the downregulation of T cell activity. In particular, such a compound can include an inhibitor of CTLA-4. An inhibitor of CTLA-4 includes any compound capable of inhibiting the activity of CTLA-4 and/or inhibiting the binding of CTLA-4 to its natural ligand (e.g., B7).

Preferably, an inhibitor of CTLA-4 includes, but is not limited to a ligand of CTLA-4 or an analog (i.e., antagonist) of CTLA-4. Preferred ligands of CTLA-4 include: an antibody that specifically binds to CTLA-4 in such a manner that CTLA-4 activity is inhibited; at least a portion of a B7 molecule, in particular a B7 fusion protein; or a synthetic oligonucleotide that binds CTLA-4 protein. A preferred analog of CTLA-4 includes a molecule capable of binding to B7 in such a manner that B7 signal transduction is not activated and CTLA-4 binding to the B7 molecule is inhibited. It is within the scope of the invention that a CTLA-4 inhibitor can comprise a nucleic acid molecule, a protein or a synthetic chemical molecule when combined in a composition of the present invention.

In another embodiment of the present invention, a therapeutic composition further comprises a pharmaceutically acceptable carrier. As used herein, a "carrier" refers to any substance suitable as a vehicle for delivering a nucleic acid molecule of the present invention to a suitable *in vivo* or *in vitro* site. As such, carriers can act as a pharmaceutically acceptable excipient of a therapeutic composition containing a nucleic acid molecule of the present invention. Preferred carriers are capable of maintaining a nucleic acid molecule of the present invention in a form that, upon arrival of the nucleic acid molecule to a cell, the nucleic acid molecule is capable of entering the cell and being expressed by the cell. Carriers of the present invention include: (1) excipients

or formularies that transport, but do not specifically target a nucleic acid molecule to a cell (referred to herein as non-targeting carriers); and (2) excipients or formularies that deliver a nucleic acid molecule to a specific site in an animal or a specific cell (i.e., targeting carriers). Examples of non-targeting carriers include, but are not limited to water, phosphate buffered saline, Ringer's solution, dextrose solution, serum-containing solutions, Hank's solution, other aqueous physiologically balanced solutions, oils, esters and glycols. Aqueous carriers can contain suitable auxiliary substances required to approximate the physiological conditions of the recipient, for example, by enhancing chemical stability and isotonicity.

Suitable auxiliary substances include, for example, sodium acetate, sodium chloride, sodium lactate, potassium chloride, calcium chloride, and other substances used to produce phosphate buffer, Tris buffer, and bicarbonate buffer. Auxiliary substances can also include preservatives, such as thimerosal, m- and o-cresol, formalin and benzol alcohol. Preferred auxiliary substances for aerosol delivery include surfactant substances non-toxic to an animal, for example, esters or partial esters of fatty acids containing from about six to about twenty-two carbon atoms. Examples of esters include, caproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, olesteric, and oleic acids. Other carriers can include metal particles (e.g., gold particles) for use

with, for example, a biolistic gun through the skin. Therapeutic compositions of the present invention can be sterilized by conventional methods and/or lyophilized.

Targeting carriers are herein referred to as "delivery vehicles." Delivery vehicles of the present invention are capable of delivering a therapeutic composition of the present invention to a target site in an animal. A "target site" refers to a site in an animal to which one desires to deliver a therapeutic composition. For example, a target site can be a malignant tumor cell, a non-malignant tumor cell, a lymph node or a lesion caused by an infectious agent, or an area around such cell, tumor or lesion, which is targeted by direct injection or delivery using liposomes or other delivery vehicles. Examples of delivery vehicles include, but are not limited to, artificial and natural lipid-containing delivery vehicles. Natural lipid-containing delivery vehicles include cells and cellular membranes. Artificial lipid-containing delivery vehicles include liposomes and micelles. A delivery vehicle of the present invention can be modified to target to a particular site in an animal, thereby targeting and making use of a nucleic acid molecule of the present invention at that site. Suitable modifications include manipulating the chemical formula of the lipid portion of the delivery vehicle and/or introducing into the vehicle a compound capable of specifically targeting a delivery vehicle to a preferred site, for example, a preferred cell type. Specifically targeting refers to causing a delivery vehicle

to bind to a particular cell by the interaction of the compound in the vehicle to a molecule on the surface of the cell. Suitable targeting compounds include ligands capable of selectively (i.e., specifically) binding another molecule at a particular site. Examples of such ligands include antibodies, antigens, receptors and receptor ligands. For example, an antibody specific for an antigen found on the surface of a cancer cell can be introduced to the outer surface of a liposome delivery vehicle so as to target the delivery vehicle to the cancer cell. Tumor cell ligands include ligands capable of binding to a molecule on the surface of a tumor cell. Manipulating the chemical formula of the lipid portion of the delivery vehicle can modulate the extracellular or intracellular targeting of the delivery vehicle. For example, a chemical can be added to the lipid formula of a liposome that alters the charge of the lipid bilayer of the liposome so that the liposome fuses with particular cells having particular charge characteristics.

A preferred delivery vehicle of the present invention is a liposome. A liposome is capable of remaining stable in an animal for a sufficient amount of time to deliver a nucleic acid molecule of the present invention to a preferred site in the animal. A liposome of the present invention is preferably stable in the animal into which it has been administered for at least about 30 minutes, more preferably for at least about 1 hour and even more preferably for at least about 24 hours.

A liposome of the present invention comprises a lipid composition that is capable of targeting a nucleic acid molecule of the present invention to a particular, or selected, site in an animal. Preferably, the lipid composition of the liposome is capable of targeting to any organ of an animal, more preferably to the lung, liver, spleen, heart brain, lymph nodes and skin of an animal, and even more preferably to the lung of an animal.

A liposome of the present invention comprises a lipid composition that is capable of fusing with the plasma membrane of the targeted cell to deliver a nucleic acid molecule into a cell. Preferably, the transfection efficiency of a liposome of the present invention is at least about 0.5 microgram (μg) of DNA per 16 nanomole (nmol) of liposome delivered to about 10^6 cells, more preferably at least about 1.0 μg of DNA per 16 nmol of liposome delivered to about 10^6 cells, and even more preferably at least about 2.0 μg of DNA per 16 nmol of liposome delivered to about 10^6 cells.

A preferred liposome of the present invention is between about 100 and about 500 nanometers (nm), more preferably between about 150 and about 450 nm and even more preferably between about 200 and about 400 nm in diameter.

Suitable liposomes for use with the present invention include any liposome. Preferred liposomes of the present invention include those liposomes standardly used in, for example, gene delivery methods known to those of skill in the art. More preferred liposomes comprise liposomes

having a polycationic lipid composition and/or liposomes having a cholesterol backbone conjugated to polyethylene glycol. Even more preferred liposomes include liposomes produced according to the method described in Example 2.

In one embodiment, a liposome of the present invention comprises a compound capable of targeting the liposome to a tumor cell. Such a liposome preferably includes a tumor cell ligand exposed on the outer surface of the liposome.

Complexing a liposome with a nucleic acid molecule of the present invention can be achieved using methods standard in the art (see, for example, methods described in Example 2). A suitable concentration of a nucleic acid molecule of the present invention to add to a liposome includes a concentration effective for delivering a sufficient amount of nucleic acid molecule to a cell such that the cell can produce sufficient superantigen and/or cytokine protein to regulate effector cell immunity in a desired manner. Preferably, nucleic acid molecules are combined with liposomes at a ratio of from about 0.1 μg to about 10 μg of nucleic acid molecule of the present invention per about 8 nmol liposomes, more preferably from about 0.5 μg to about 5 μg of nucleic acid molecule per about 8 nmol liposomes, and even more preferably about 1.0 μg of nucleic acid molecule per about 8 nmol liposomes.

Another preferred delivery vehicle comprises a recombinant virus particle vaccine. A recombinant virus particle vaccine of the present invention includes a therapeutic composition of the present invention, in which

the recombinant molecules contained in the composition are packaged in a viral coat that allows entrance of DNA into a cell so that the DNA is expressed in the cell. A number of recombinant virus particles can be used, including, but not limited to, those based on alphaviruses, poxviruses, adenoviruses, herpesviruses, arena virus and retroviruses.

Another preferred delivery vehicle comprises a recombinant cell vaccine. Preferred recombinant cell vaccines of the present invention include tumor vaccines, in which allogeneic (i.e., cells derived from a source other than a patient, but that are histiotype compatible with the patient) or autologous (i.e., cells isolated from a patient) tumor cells are transfected with recombinant molecules contained in a therapeutic composition, irradiated and administered to a patient by, for example, intradermal, intravenous or subcutaneous injection. Therapeutic compositions to be administered by tumor cell vaccine, include recombinant molecules of the present invention without carrier. Tumor cell vaccine treatment is useful for the treatment of both tumor and metastatic cancer. Use of a tumor vaccine of the present invention is particular useful for treating metastatic cancer, including preventing metastatic disease, as well as, curing existing metastatic disease. Methods for developing and administering include those standard in the art (see for example, Dranoff et al., *Proc. Natl. Acad. Sci. USA* 90:3539-3543, 1993, which is incorporated herein by reference in its entirety).

A therapeutic composition of the present invention is useful for the treatment of a variety of diseases, including, but not limited to, cancer, autoimmune disease, infectious diseases, and other diseases that can be alleviated by either stimulating or suppressing T cell activity. As used herein, the term "treatment" refers to protecting an animal from a disease or alleviating a disease in an animal. A therapeutic composition of the present invention is advantageous for the treatment of cancer in that the composition overcomes the mechanisms by which cancer cells avoid immune elimination (i.e., by which cancer cells avoid the immune response effected by the animal in response to the disease). Cancer cells can avoid immune elimination by, for example, being only slightly immunogenic, modulating cell surface antigens and inducing immune suppression. Suitable therapeutic compositions for use in the treatment of cancer comprises a superantigen-encoding recombinant molecule; or a combination of a superantigen-encoding recombinant molecule, with a cytokine-encoding recombinant molecule and/or a chemokine recombinant molecule of the present invention. Preferred therapeutic compositions for use in the treatment of cancer comprises a superantigen-encoding recombinant molecule; or a combination of a superantigen-encoding recombinant molecule with a cytokine-encoding recombinant molecule and/or a chemokine recombinant molecule of the present invention combined (separately or together) with a delivery vehicle, preferably a liposome, such as disclosed herein.

A therapeutic composition of the present invention, upon entering targeted cells, leads to the production of superantigen, cytokine and/or chemokine protein that activate cytotoxic T cells, natural killer cells, T helper cells and macrophages. Such cellular activation overcomes the otherwise relative lack of immune response to cancer cells, leading to the destruction of such cells.

A therapeutic composition of the present invention is useful for the treatment of cancers, both tumors and metastatic forms of cancer. Treatment with the therapeutic composition overcomes the disadvantages of traditional treatments for metastatic cancers. For example, compositions of the present invention can target dispersed metastatic cancer cells that cannot be treated using surgical methods. In addition, administration of such compositions do not result in the harmful side effects caused by chemotherapy and radiation therapy.

A therapeutic composition of the present invention is preferably used to treat cancers, including, but not limited to, melanomas, squamous cell carcinoma, breast cancers, head and neck carcinomas, thyroid carcinomas, soft tissue sarcomas, bone sarcomas, testicular cancers, prostatic cancers, ovarian cancers, bladder cancers, skin cancers, brain cancers, angiosarcomas, hemangiosarcomas, mast cell tumors, primary hepatic cancers, lung cancers, pancreatic cancers, gastrointestinal cancers, renal cell carcinomas, hematopoietic neoplasias, leukemias and lymphomas. Particularly preferred cancers to treat with a

therapeutic composition of the present invention, include melanomas, lung cancers, thyroid carcinomas, breast cancers, renal cell carcinomas, squamous cell carcinomas, brain tumors and skin cancers. A therapeutic composition of the present invention is useful for treating tumors that can form in such cancers, including malignant and benign tumors.

A therapeutic composition of the present invention is also advantageous for the treatment of infectious diseases as a long term, targeted therapy for primary lesions (e.g., granulomas) resulting from the propagation of a pathogen. As used herein, the term "lesion" refers to a lesion formed by infection of an animal with a pathogen. Preferred therapeutic compositions for use in the treatment of an infectious disease comprise a superantigen-encoding recombinant molecule; or a combination of a superantigen-encoding recombinant molecule, with a cytokine-encoding recombinant molecule and/or a chemokine recombinant molecule of the present invention. More preferred therapeutic compositions for use in the treatment of infectious disease comprise a superantigen-encoding recombinant molecule; or a combination of superantigen-encoding recombinant molecule, with a cytokine-encoding recombinant molecule and/or a chemokine recombinant molecule of the present invention combined with a delivery vehicle, preferably a liposome of the present invention. Similar to the mechanism described for the treatment of cancer, treatment of infectious diseases with superantigen,

cytokine and/or chemokine can result in increased T cell, natural killer cell, and macrophage cell activity that overcome the relative lack of immune response to a lesion formed by a pathogen.

A therapeutic composition of the present invention is particularly useful for the treatment of infectious diseases caused by pathogens, including, but not limited to, intracellular bacteria (i.e., a bacteria that resides in a host cell), internal parasites, pathogenic fungi and endoparasites. Particularly preferred infectious diseases to treat with a therapeutic composition of the present invention include tuberculosis, leprosy, aspergillosis, coccidioidomycosis, cryptococcoses, leishmaniasis and toxoplasmosis.

In order to treat an animal with disease, a therapeutic composition of the present invention is administered to the animal in an effective manner such that the composition is capable of treating that animal from disease. For example, a recombinant molecule, when administered to an animal in an effective manner, is able to stimulate effector cell immunity in a manner that is sufficient to alleviate the disease afflicting the animal. According to the present invention, treatment of a disease refers to alleviating a disease and/or preventing the development of a secondary disease resulting from the occurrence of a primary disease.

An effective administration protocol (i.e., administering a therapeutic composition in an effective

manner) comprises suitable dose parameters and modes of administration that result in treatment of a disease. Effective dose parameters and modes of administration can be determined using methods standard in the art for a particular disease. Such methods include, for example, determination of survival rates, side effects (i.e., toxicity) and progression or regression of disease. In particular, the effectiveness of dose parameters and modes of administration of a therapeutic composition of the present invention when treating cancer can be determined by assessing response rates. Such response rates refer to the percentage of treated patients in a population of patients that respond with either partial or complete remission. Remission can be determined by, for example, measuring tumor size or microscopic examination for the presence of cancer cells in a tissue sample.

In accordance with the present invention, a suitable single dose size is a dose that is capable of treating an animal with disease when administered one or more times over a suitable time period. Doses can vary depending upon the disease being treated. In the treatment of cancer, a suitable single dose can be dependent upon whether the cancer being treated is a primary tumor or a metastatic form of cancer. Doses of a therapeutic composition of the present invention suitable for use with direct injection techniques can be used by one of skill in the art to determine appropriate single dose sizes for systemic administration based on the size of an animal. A suitable

single dose of a therapeutic composition to treat a tumor is a sufficient amount of a superantigen-encoding recombinant molecule; or a superantigen-encoding recombinant molecule, with a cytokine-encoding recombinant molecule and/or a chemokine recombinant molecule to reduce, and preferably eliminate, the tumor following transfection of the recombinant molecules into cells at or near the tumor site. A preferred single dose of the superantigen-encoding recombinant molecule is an amount that, when transfected into a target cell population, leads to the production of from about 250 femtograms (fg) to about 1 μg, preferably from about 500 fg to about 500 picogram (pg), and more preferably from about 1 pg to about 100 pg of superantigen per transfected cell. A preferred single dose of a cytokine-encoding recombinant molecule is an amount that, when transfected into a target cell population, leads to the production of from about 10 pg to about 1 μg, preferably from about 100 pg to about 750 pg, and more preferably about 500 pg of cytokine per transfectant. A preferred single dose of a chemokine-encoding recombinant molecule is an amount that, when transfected into a target cell population, leads to the production of from about 1 fg to about 1 μg, preferably from about 1 pg to about 10 ng, and more preferably from about 1 pg to about 1 ng chemokine per transfectant.

A suitable single dose of a superantigen-encoding recombinant molecule; or a combination of a superantigen-encoding recombinant molecule, with a cytokine-encoding

recombinant molecule and/or a chemokine-encoding recombinant molecule in a non-targeting carrier to administer to an animal to treat a tumor, is an amount capable of reducing, and preferably eliminating, the tumor following transfection of the recombinant molecules into cells at or near the tumor site. A preferred single dose of a therapeutic composition to treat a tumor is from about 100 μg to about 2 milligrams (mg) of total recombinant molecules, more preferably from about 150 μg to about 1 mg of total recombinant molecules, and even more preferably from about 200 μg to about 800 μg of total recombinant molecules. A preferred single dose of a superantigen-encoding recombinant molecule complexed with liposomes, is from about 100 μg of total DNA per 800 nmol of liposome to about 2 mg of total recombinant molecules per 16 micromole (μmol) of liposome, more preferably from about 150 μg per 1.2 μmol of liposome to about 1 mg of total recombinant molecules per 8 μmol of liposome, and even more preferably from about 200 μg per 2 μmol of liposome to about 400 μg of total recombinant molecules per 3.2 μmol of liposome.

A preferred single dose of a cytokine-encoding recombinant molecule or a chemokine-encoding recombinant molecule in a non-targeting carrier to administer to an animal to treat a tumor, is from about 100 μg to about 2 mg of total recombinant molecules, more preferably from about 150 μg to about 1 mg of total recombinant molecules, and even more preferably from about 200 μg to about 400 μg of total recombinant molecules. A preferred single dose of a

cytokine-encoding recombinant molecule or a chemokine-encoding recombinant molecule complexed with liposomes to administer to an animal to treat a tumor, is from about 100 μg of total recombinant molecules per 800 nmol of liposome to about 2 mg of total recombinant molecules per 16 μmol of liposome, more preferably from about 150 μg per 1.2 μmol of liposome to about 1 mg of total recombinant molecules per 8 μmol of liposome, and even more preferably from about 200 μg per 2 μmol of liposome to about 400 μg of total recombinant molecules per 6.4 μmol of liposome.

A preferred single dose of a superantigen-encoding recombinant molecule in a non-targeting carrier to administer to an animal treat a metastatic cancer, is from about 100 μg to about 4 mg of total recombinant molecules, more preferably from about 150 μg to about 3 mg of total recombinant molecules, and even more preferably from about 200 μg to about 2 mg of total recombinant molecules. A preferred single dose of a superantigen-encoding recombinant molecule complexed with liposomes to administer to an animal to treat a metastatic cancer, is from about 100 μg of total recombinant molecules per 800 nmol of liposome to about 4 mg of total recombinant molecules per 32 μmol of liposome, more preferably from about 200 μg per 1.6 μm of liposome to about 3 mg of total recombinant molecules per 24 μmol of liposome, and even more preferably from about 400 μg per 3.2 μmol of liposome to about 2 mg of total recombinant molecules per 16 μmol of liposome.

A preferred single dose of a cytokine-encoding recombinant molecule or a chemokine-encoding recombinant molecule in a non-targeting carrier to administer to an animal to treat a metastatic cancer, is from about 100 μg to about 4.0 mg of total recombinant molecules, more preferably from about 150 μg to about 3 mg of total recombinant molecules, and even more preferably from about 200 μg to about 2 mg of total recombinant molecules. A preferred single dose of a cytokine-encoding recombinant molecule or a chemokine-encoding recombinant molecule complexed with liposomes to administer to an animal to treat a metastatic cancer, is from about 100 μg of total recombinant molecules per 800 nmol of liposome to about 4.0 mg of total recombinant molecules per 32 μmol of liposome, more preferably from about 200 μg per 1.6 μmol of liposome to about 3 mg of total recombinant molecules per 24 μmol of liposome, and even more preferably from about 400 μg per 3.2 μmol of liposome to about 2 μg of total recombinant molecules per 16 μmol of liposome.

According to the present invention, a single dose of a therapeutic composition useful to treat a lesion, comprising a superantigen-encoding recombinant molecule in a non-targeting carrier or liposomes, respectively, and a cytokine-encoding recombinant molecule in a non-targeting carrier or liposomes, respectively, is substantially similar to those doses used to treat a tumor (as described in detail above).

The number of doses administered to an animal is dependent upon the extent of the disease and the response of an individual patient to the treatment. For example, a large tumor may require more doses than a smaller tumor. In some cases, however, a patient having a large tumor may require fewer doses than a patient with a smaller tumor, if the patient with the large tumor responds more favorably to the therapeutic composition than the patient with the smaller tumor. Thus, it is within the scope of the present invention that a suitable number of doses includes any number required to cause regression of a disease. A preferred protocol is monthly administrations of single doses (as described above) for up to about 1 year. A preferred number of doses of a therapeutic composition comprising a superantigen-encoding recombinant molecule; or a combination of a superantigen-encoding recombinant molecule, with a cytokine-encoding recombinant molecule and/or a chemokine-encoding recombinant molecule in a non-targeting carrier or complexed with liposomes in order to treat a tumor is from about 1 to about 10 administrations per patient, preferably from about 2 to about 8 administrations per patient, and even more preferably from about 3 to about 5 administrations per patient. Preferably, such administrations are given once every 2 weeks until signs of remission appear, then once a month until the disease is gone.

A preferred number of doses of a therapeutic composition comprising a superantigen-encoding recombinant

molecule; or a combination of a superantigen-encoding recombinant molecule, with a cytokine-encoding recombinant molecule and/or a chemokine-encoding recombinant molecule in a non-targeting carrier or complexed with liposomes in order to treat a metastatic cancer, is from about 2 to about 10 administrations patient, more preferably from about 3 to about 8 administrations per patient, and even more preferably from about 3 to about 7 administrations per patient. Preferably, such administrations are given once every 2 weeks until signs of remission appear, then once a month until the disease is gone.

According to the present invention, the number of doses of a therapeutic composition to treat a lesion comprising a superantigen-encoding recombinant molecule; or a combination of a superantigen-encoding recombinant molecule, with a cytokine-encoding recombinant molecule and/or a chemokine-encoding recombinant molecule, in a non-targeting carrier or liposomes, respectively, is substantially similar to those number of doses used to treat a tumor (as described in detail above).

A therapeutic composition is administered to an animal in a fashion to enable expression of an introduced recombinant molecule of the present invention into a curative protein in the animal to be treated for disease. A therapeutic composition can be administered to an animal in a variety of methods including, but not limited to, local administration of the composition into a site in an animal. Examples of such sites include lymph nodes, a site

that contains abnormal cells or pathogens to be destroyed (e.g., injection locally within the area of a tumor or a lesion); and systemic administration.

Therapeutic compositions to be delivered by local administration include: (a) recombinant molecules of the present invention in a non-targeting carrier (e.g., as "naked" DNA molecules, such as is taught, for example in Wolff et al., 1990, *Science 247*, 1465-1468); and (b) recombinant molecules of the present invention complexed to a delivery vehicle of the present invention. Suitable delivery vehicles for local administration comprise liposomes. Delivery vehicles for local administration can further comprise ligands for targeting the vehicle to a particular site (as described in detail herein).

A preferred method of local administration is by direct injection. Direct injection techniques are particularly useful for the treatment of disease by, for example, injecting the composition into a mass formed by abnormal cells, a lymph node or a granuloma mass induced by pathogens. Preferably, a recombinant molecule of the present invention complexed with a delivery vehicle is administered by direct injection into or locally within the area of a tumor mass, a lymph node, a granuloma mass or a cancer cell. Administration of a composition locally within an area of a mass or a cell refers to injecting the composition centimeters and preferably, millimeters within the mass or the cell. A preferred tumor mass to inject includes discrete inner body and cutaneous solid tumors.

A preferred inner body tumor to inject includes a discrete solid tumor that forms in the brain, breast, liver, kidney, colon, prostate, testicular, ovary, spleen and/or lymph node. A preferred cutaneous tumor to inject includes a discrete solid melanoma.

A preferred lymph node to inject includes a draining lymph node that "drains" a site containing abnormal cells or pathogens. As used herein, the term "draining lymph node" refers to a lymph node that is located downstream of a site containing abnormal cells or pathogens is based on the direction of the lymphatic flow of an animal (see general discussion in Hole, *Human Anatomy and Physiology*, Edward G. Jaffe, ed., Wm. C Brown Publishers, Dubuque, IA; and G.C. Christiansen et al., *Anatomy of the Dog*, W.B. Saunders Publishers, Philadelphia, PN, 1979; both of which are incorporated herein by this reference). A preferred draining lymph node to inject comprises the draining lymph node most proximal to a site containing abnormal cells or pathogens. Thus, a skilled artisan can choose the site of lymph node injection based upon the location of the site containing abnormal cells or pathogens. Examples of lymph nodes to injection include: the mandibular lymph node if a tumor is located in the oral cavity; and the superficial cervical lymph node of a tumor is located in the front leg region. Effector cells travel from a site containing abnormal cells or pathogens. Injection of a therapeutic composition of the present invention into a lymph node can result in expression of a superantigen, a cytokine and/or

a chemokine by an effector cell from the lymph node or that has drained into the lymph node. Such expression can result in the activation of T lymphocytes, which can travel back to the site containing abnormal cells or pathogens to enhance the immune response at the site.

Another method of local administration is to contact a therapeutic composition of the present invention in or around a surgical wound. For example, a patient can undergo surgery to remove a tumor. Upon removal of the tumor, the therapeutic composition can be coated on the surface of tissue inside the wound or the composition can be injected into areas of tissue inside the wound. Such local administration is useful for treating cancer cells not excised by the surgical procedure, as well as, preventing recurrence of the primary tumor or development of a secondary tumor in the area of the surgery.

In one embodiment, a therapeutic composition of the present invention can be introduced to a tumor cell *in vivo*. In another embodiment, a therapeutic composition of the present invention can be introduced to a non-tumor cell *in vivo* or *in vitro*. Methods to introduce a therapeutic composition *in vivo* are disclosed herein. Methods to introduce a therapeutic composition *in vitro* include methods standard in the art, such as culturing cells in the presence of a therapeutic composition for a sufficient amount of time to enable a nucleic acid molecule of the present invention to pass through the plasma membrane in a cell and subsequently to be expressed in the cell.

Therapeutic compositions useful in systemic administration, include recombinant molecules of the present invention complexed to a targeted delivery vehicle of the present invention. Suitable delivery vehicles for use with systemic administration comprise liposomes comprising ligands for targeting the vehicle to a particular site, preferably ligands for targeting the vehicle to a site of a cancer or a lesion (depending upon the disease being treated). For cancer treatment, ligands capable of selectively binding to a cancer cell or to a cell within the area of a cancer cell are preferred. Systemic administration is useful for the treatment of both tumor and metastatic cancer and systemic infectious diseases. Systemic administration is particularly useful for the treatment of metastatic forms of cancer, in which the cancer cells are dispersed (i.e., not localized within a single tumor mass). Systemic administration is particularly advantageous when organs, in particular difficult to reach organs (e.g., heart, spleen, lung or liver) are the targeted sites of treatment.

Preferred methods of systemic administration, include intravenous injection, aerosol, oral and percutaneous (topical) delivery. Intravenous injections can be performed using methods standard in the art. Aerosol delivery can also be performed using methods standard in the art (see, for example, Stribling et al., *Proc. Natl. Acad. Sci. USA* 189:11277-11281, 1992, which is incorporated herein by reference in its entirety). Oral delivery can be

performed by complexing a therapeutic composition of the present invention to a carrier capable of withstanding degradation by digestive enzymes in the gut of an animal. Examples of such carriers, include plastic capsules or tablets, such as those known in the art. Topical delivery can be performed by mixing a therapeutic composition of the present invention with a lipophilic reagent (e.g., DMSO) that is capable of passing into the skin.

Therapeutic compositions of the present invention can be administered to any animal, preferably to mammals and birds, and more preferably to humans, house pets, economic produce animals and zoo animals. Economic produce animals include animals to be consumed or that produce useful products (e.g., sheep for wool production). Zoo animals include those animals harbored in zoos. Preferred animals to protect include humans, dogs, cats, sheep, cattle, horses and pigs, with humans and dogs being particularly preferred. While a therapeutic composition of the present invention is effective to treat disease in inbred species of animals, the composition is particularly useful for treating outbred species of animals, in particular those having tumors.

Yet another embodiment of the present invention is a method to suppress T cell activity in an animal, the method comprising administering to an animal an effective amount of a therapeutic composition comprising: (a) a naked nucleic acid molecule encoding a superantigen; and (b) a pharmaceutically acceptable carrier, in which the nucleic

acid molecule is operatively linked to a transcription control sequence, and in which the therapeutic composition is targeted to a site in the animal that contains excessive T cell activity.

Suitable embodiments, single dose sizes, number of doses and modes of administration of a therapeutic composition of the present invention useful in a treatment method of the present invention are disclosed in detail herein.

A therapeutic composition of the present invention is also advantageous for the treatment of autoimmune diseases in that the composition suppresses the harmful stimulation of T cells by autoantigens (i.e., a "self", rather than a foreign antigen). Superantigen-encoding recombinant molecules in a therapeutic composition, upon transfection into a cell, produce superantigens that delete harmful populations of T cells involved in an autoimmune disease. A preferred therapeutic composition for use in the treatment of autoimmune disease comprises a superantigen-encoding recombinant molecule of the present invention. A more preferred therapeutic composition for use in the treatment of autoimmune disease comprises a superantigen-encoding recombinant molecule combined with a non-targeting carrier of the present invention, preferably saline or phosphate buffered saline. Such a therapeutic composition of the present invention is particularly useful for the treatment of autoimmune diseases, including but not limited to, multiple sclerosis, systemic lupus

erythematosus, myasthenia gravis, rheumatoid arthritis, insulin dependent diabetes mellitus, psoriasis, polyarteritis, immune mediated vasculitides, immune mediated glomerulonephritis, inflammatory neuropathies and sarcoidosis.

A single dose of a superantigen-encoding nucleic acid molecule in a non-targeting carrier to administer to an animal to treat an autoimmune disease is from about 0.1 μg to about 200 μg of total recombinant molecules per kilogram (kg) of body weight, more preferably from about 0.5 μg to about 150 μg of total recombinant molecules per kg of body weight, and even more preferably from about 1 μg to about 10 μg of total recombinant molecules per kg of body weight.

The number of doses of a superantigen-encoding recombinant molecule in a non-targeting carrier to be administered to an animal to treat an autoimmune disease is an injection about once every 6 months, more preferably about once every 3 months, and even more preferably about once a month.

A preferred method to administer a therapeutic composition of the present invention to treat an autoimmune disease is by local administration, preferably direct injection. Direct injection techniques are particularly important in the treatment of an autoimmune disease. Preferably, a therapeutic composition is injected directly into muscle cells in a patient, which results in prolonged expression (e.g., weeks to months) of a recombinant molecule of the present invention. Preferably, a

recombinant molecule of the present invention in the form of "naked DNA" is administered by direct injection into muscle cells in a patient.

Another aspect of the present invention is an adjuvant for use with a nucleic acid-based vaccine to protect an animal from a disease or a remedy to treat a diseased animal. Adjuvants of the present invention comprise: (a) a superantigen-encoding nucleic acid molecule of the present invention; or (b) a combination of a superantigen-encoding nucleic acid molecule of the present invention with a cytokine nucleic acid molecule of the present invention, a chemokine nucleic acid molecule of the present invention or mixtures thereof.

Suitable compounds to combine with an adjuvant of the present invention, to form an adjuvant composition (i.e., a vaccine composition useful as a preventative therapeutic reagent or a therapeutic remedy useful to alleviate a disease) of the present invention, include any compound that is administered to an animal as an immunogen. As used herein, an immunogen of the present invention comprises a compound capable of eliciting an immune response in an animal. Preferably, an immunogen of the present invention is derived from a foreign agent including a pathogen. Also preferably, an immunogen of the present invention includes an allergen (organic or inorganic), tumor antigens and self-antigens.

A preferred immunogen is derived from a pathogen including, but not limited to, a virus, a bacteria, a

eukaryotic parasite and unicellular protozoa (e.g., amoeba). Preferred eukaryotic parasites include protozoan parasites, helminth parasites (such as nematodes, cestodes, trematodes, ectoparasites and fungi.

A preferred immunogen also includes an allergen including, but not limited to, a plant allergen, an animal allergen, a bacterial allergen, a parasitic allergen, a metal-based allergen that causes contact sensitivity and inorganic allergens such as silica, beryllium, xenobiotics, synthetic drugs and dyes. A more preferred allergen includes weed, grass, tree, peanut, mite, flea, cat, house dust and bacterial products antigens.

A preferred immunogen derived from a bacteria includes an immunogen that protects an animal from or alleviates *Mycobacterium* infection, in particular *M. tuberculosis*, *M. leprae*, *M. avium*, and/or *M. bovis* infection. A more preferred bacterial immunogen of the present invention includes a peptide, mimetopes thereof and compositions containing the same, as disclosed in U.S. Patent Serial No. 08/484,169, filed June 7, 1995, which is incorporated herein by this reference. In one embodiment, an immunogen comprises a nucleic acid molecule encoding an immunogenic protein. Such immunogen-encoding nucleic acid molecules can be designed by those of skill in the art based upon the amino acid sequence of the immunogen. In addition, a recombinant molecule encoding an immunogen of the present invention can be produced using the recombinant DNA technology disclosed herein and known to those of skill

in the art. In other embodiments, an immunogen can comprise a peptide, a polypeptide or a chemical compound as disclosed herein. All such embodiments of an immunogen are useful with an adjuvant of the present invention.

In order to treat an animal (i.e., vaccinate or remedy), an adjuvant composition of the present invention is administered to the animal in an effective manner such that the composition is capable of protecting an animal from or alleviating a disease. For example, an adjuvant, when administered to an animal in an effective manner, is able to stimulate effector cell immunity in a manner that is sufficient to prevent an initial or continued disease response by the subject animal.

An effective administration protocol (i.e., administering an adjuvant composition in an effective manner) comprises suitable dose parameters, and modes and times of administration that result in the treatment of an animal. Effective dose parameters and modes of administration can be determined using methods standard in the art for a particular adjuvant composition. Such methods include, for example: determination of side effects (i.e., toxicity) of an adjuvant composition; progression of a disease upon administration of an adjuvant composition; magnitude and/or duration of antibody response by an animal against an immunogen contained in an adjuvant composition; magnitude and/or duration of a cell mediated immune response in an animal against an adjuvant composition; similarity of an immune response to an adjuvant composition

in different species of animals; and/or effect of breed (in non-human animals) or race (in humans) on responsiveness to an adjuvant composition. In particular, the effectiveness of dose parameters and modes of administration of an adjuvant composition of the present invention can be determined by assessing antibody production *in vivo*, skin test sensitivities *in vivo*, cytokine production *in vitro*, antigen-specific proliferation *in vitro*, cytotoxic T cell activity *in vitro*, reduction of tumor burden *in vivo* and/or reduction of infectious agent burden *in vivo*. Tests standard in the art can be used to determine antibody production (e.g., enzyme-linked immunoassays), skin test sensitivities (e.g., subcutaneous injection of an immunogen into a vaccinated animal to assess weal formation, induration and erythema), cytokine production (e.g., immunoassays using cytokine-specific antibodies or bio-assays using cytokine-dependent cell lines), antigen-specific proliferation (e.g., ^{3}H-thymidine incorporation), cytotoxic T cell activity (e.g., measure release of ^{51}Cr from target cells), reduction of tumor burden (e.g., measure size of a tumor) and/or reduction of infectious agent burden (e.g., obtaining, for example, viral titers, bacterial colony counts or parasite counts).

An effective dose refers to a dose capable of immunizing an animal against an immunogen. Effective doses can vary depending upon, for example, the adjuvant used, the immunogen being administered, and the size and type of the recipient animal. Effective doses to treat an animal

to an immunogen include doses administered over time that are capable of preventing or alleviating a disease in an animal to, for example, a pathogen or allergen. For example, a first treatment dose can comprise an amount of an adjuvant composition of the present invention that causes a minimal hypersensitive response when administered to a hypersensitive animal. A second treatment dose can comprise a greater amount of the same adjuvant composition than the first dose. Effective treatment doses can comprise increasing concentrations of the adjuvant composition necessary to treat an animal such that the animal does not exhibit signs of a disease.

In accordance with the present invention, a suitable single dose is a dose that is capable of vaccinating an animal against a foreign agent when administered one or more times over a suitable time period. For example, a preferred single dose of an adjuvant composition of the present invention is from about 100 μg to about 1 mg of the adjuvant composition per kilogram body weight of the animal. Further treatments with the adjuvant composition can be administered from about 1 week to about 1 year after the original administration. Further treatments with the adjuvant composition preferably are administered when the animal is no longer protected from an immunogen to which the animal has been treated. Particular administration doses and schedules can be developed by one of skill in the art based upon the parameters discussed above.

The number of doses administered to an animal is dependent upon the immunogen and the response of an individual patient to the adjuvant composition. For example, treatment of one strain of virus may require more doses than treatment of a more immunogenic strain of virus. Thus, it is within the scope of the present invention that a suitable number of doses includes any number required to treat an animal. A preferred number of doses of an adjuvant composition comprising a superantigen-encoding recombinant molecule, and/or a cytokine-encoding recombinant molecule and/or a chemokine-encoding recombinant molecule is from about 2 to about 20 administrations, preferably from about 3 to about 10 administrations, and even more preferably from about 3 to about 5 administrations per patient per year. Preferably, such administrations are given once every 2 weeks until, for example, antibody production against an immunogen increases or decreases, cell mediated immunity increases, and/or a clinical response is observed when an adjuvant composition is administered as a therapeutic remedy.

A preferred single dose of the superantigen-encoding recombinant molecule is an amount that, when transfected into a muscle cells, skin tissue, lung cells or other suitable cellular sites, leads to the production of from about 10 femtograms (fg) to about .01 μg, preferably from about 100 fg to about 1 picogram (pg), and more preferably from about 1 pg to about 5 pg of superantigen per transfected cell. A preferred single dose of a cytokine-

encoding recombinant molecule is an amount that when transfected into a target cell population leads to the production of from about 10 pg to about .01 μg, preferably from about 100 fg to about 2 pg, and more preferably about 1 pg of cytokine per transfected. A preferred single dose of a chemokine-encoding recombinant molecule is an amount that when transfected into a target cell population leads to the production of from about 1 pg to about .01 μg, preferably from about 0.1 pg to about 10 pg, and more preferably about 1 pg of chemokine per transfected.

In one embodiment, an adjuvant composition of the present invention comprises up to about 50% of an immunogen-encoding recombinant molecule and up to about 50% of a superantigen-encoding recombinant molecule. Preferably, an adjuvant composition of the present invention comprises no more than about 1.5 mg of immunogen-encoding recombinant molecule and no more than about 1.5 mg of superantigen-encoding recombinant molecule, more preferably no more than about 1 mg of immunogen-encoding recombinant molecule and no more than about 1 mg of superantigen-encoding recombinant molecule, and even more preferably no more than about 0.5 mg of immunogen-encoding recombinant molecule and no more than about 0.5 mg of superantigen-encoding recombinant molecule per animal.

In another embodiment, an adjuvant composition of the present invention comprises an immunogen-encoding recombinant molecule in an amount up to about 66% by weight of the composition and a superantigen-encoding recombinant

molecule in an amount up to about 33% by weight of the composition. Preferably, an adjuvant composition of the present invention comprises no more than about 2000 μg of immunogen-encoding recombinant molecule and no more than about 1000 μg of superantigen-encoding recombinant molecule, more preferably no more than about 1400 μg of immunogen-encoding recombinant molecule and no more than about 660 μg of superantigen-encoding recombinant molecule, and even more preferably no more than about 670 μg of immunogen-encoding recombinant molecule and no more than about 330 μg of superantigen-encoding recombinant molecule per animal.

In another embodiment, an adjuvant composition of the present invention comprises an immunogen-encoding recombinant molecule in an amount up to about 50% of the composition; a superantigen-encoding recombinant molecule in an amount up to about 25% of the composition; and a cytokine-encoding recombinant molecule or chemokine-encoding recombinant molecule or mixtures thereof, in an amount up to about 25% of the composition. According to the present embodiment, a cytokine-encoding recombinant molecule or a chemokine-encoding recombinant molecule can be used alone or in combination with each other. When used in combination, the ratio of cytokine-encoding recombinant molecule to chemokine-encoding recombinant molecule can be varied according to need. The ratio can be determined based upon the effectiveness of the adjuvant composition at

vaccinating an animal against a foreign agent using the methods and parameters disclosed herein.

In one embodiment, an adjuvant composition of the present invention comprises: no more than about 2000 μg of immunogen-encoding recombinant molecule, no more than about 500 μg of superantigen-encoding recombinant molecule, and no more than about 500 μg of cytokine-encoding recombinant molecule or no more than about 500 μg of chemokine-encoding recombinant molecule; more preferably no more than about 1400 μg of immunogen-encoding recombinant molecule, no more than about 300 μg of superantigen-encoding recombinant molecule, and no more than about 300 μg of cytokine-encoding recombinant molecule or no more than about 300 μg of chemokine-encoding recombinant molecule; and even more preferably no more than about 660 μg of immunogen-encoding recombinant molecule, no more than about 160 μg of superantigen-encoding recombinant molecule, and no more than about 160 μg of cytokine-encoding recombinant molecule or no more than about 160 μg of chemokine-encoding recombinant molecule per animal.

In another embodiment, an adjuvant composition of the present invention comprises: no more than about 2000 μg of immunogen-encoding recombinant molecule, no more than about 500 μg of superantigen-encoding recombinant molecule, and no more than about 250 μg of cytokine-encoding recombinant molecule and no more than about 250 μg of chemokine-encoding recombinant molecule; more preferably no more than about 1000 μg of immunogen-encoding recombinant molecule,

no more than about 250 μg of superantigen-encoding recombinant molecule, and no more than about 125 μg of cytokine-encoding recombinant molecule and no more than about 125 μg of chemokine-encoding recombinant molecule; and even more preferably no more than about 660 μg of immunogen-encoding recombinant molecule, no more than about 160 μg of superantigen-encoding recombinant molecule, and no more than about 80 μg of cytokine-encoding recombinant molecule and no more than about 80 μg of chemokine-encoding recombinant molecule per animal.

Adjuvant compositions are preferably delivered by intramuscular administration in the form of "naked" DNA molecules, such as disclosed herein. Preferably, an adjuvant composition of the present invention is delivered by intramuscular, intravenous, intraperitoneal and/or intraarterial injection, and/or injection directly into specific cellular locations (e.g., into a tumor). Preferred sites of intramuscular injections include caudal thigh muscle, back muscle and into the buttocks of a human.

Preferably, an adjuvant composition of the present invention comprises a suitable pharmaceutically acceptable carrier for delivering the composition intramuscularly. A preferred carrier for use with an adjuvant includes phosphate buffered saline, water, Ringer's solution, dextrose solution, Hank's balanced salt solution and normal saline. A more preferred carrier includes phosphate

buffered saline and normal saline, with phosphate buffered saline being even more preferred.

Preferably, an adjuvant composition of the present invention comprises a mixture including a superantigen encoding molecule including an SEA-encoding recombinant molecule, an SEB-encoding recombinant molecule or mixtures thereof, and an immunogen-encoding recombinant molecule of the present invention; a superantigen encoding molecule including an SEA-encoding recombinant molecule, an SEB-encoding recombinant molecule or mixtures thereof, a cytokine encoding molecule including a GM-CSF-encoding recombinant molecule and an immunogen-encoding recombinant molecule of the present invention; or a superantigen encoding molecule including an SEA-encoding recombinant molecule, an SEB-encoding recombinant molecule or mixtures thereof, a chemokine encoding molecule including a MIP1α, MIP1β, IL-8 or RANTES recombinant molecule and an immunogen-encoding recombinant molecule of the present invention.

In a preferred embodiment, an adjuvant of the present invention includes the following recombinant molecules contained in phosphate buffered saline: (1) PCR_3-SEA, PCR_3-SEA.S, PCR_3-SEB, PCR_3-SEB.S, PCR_3-TSST and mixtures thereof; (2) mixtures of up to about 50% PCR_3-SEA, PCR_3-SEA.S, PCR_3-SEB, PCR_3-SEB.S and/or PCR_3-TSST, and up to about 50% PCR_3-GM_3; (3)

mixtures of up to about 50% PCR_3-SEA, PCR_3-SEA.S, PCR_3-SEB, PCR_3-SEB.S and/or PCR_3-TSST, and up to about 50% PCR_3-MIP1α;

(4) mixtures of up to about 50% PCR_3-SEA, PCR_3-SEA.S, PCR_3-SEB, PCR_3-SEB.S and/or PCR_3-TSST, and up to about 50% PCR_3-MIP1β; (5) mixtures of up to about 50% PCR_3-SEA, PCR_3-SEA.S, PCR_3-SEB, PCR_3-SEB.S and/or PCR_3-TSST, and up to about 50% PCR_3-RANTES; (6) mixtures of up to about 50% PCR_3-SEA, PCR_3-SEA.S, PCR_3-SEB, PCR_3-SEB.S and/or PCR_3-TSST, up to about 25% PCR_3-GM_3, and up to about 25% PCR_3-MIP1α, PCR_3-MIP1β and/or PCR_3-RANTES.

According to the present invention, a preferred embodiment of an adjuvant composition of the present invention includes: (1) an immunogen-encoding recombinant molecule the present invention in an amount up to about 50% of the composition and a preferred embodiment of an adjuvant of the present invention in an amount up to about 50% of the composition; or (2) an immunogen-encoding recombinant molecule in an amount up to about 66% of the composition and a preferred embodiment of an adjuvant of the present invention in an amount up to about 33% of the composition, in phosphate buffered saline.

The following examples are provided for the purposes of illustration and are not intended to limit the scope of the present invention.

EXAMPLES

Example 1

This example describes the production of recombinant molecules encoding superantigens and cytokines.

Full-length cDNA encoding *Staphylococcal enterotoxin* B (SEB; SEQ ID NO:1) and *Staphylococcal enterotoxin* A (SEA;

SEQ ID NO:3) were produced by polymerase chain reaction (PCR) amplification using templates obtained from Dr. John Kappler (National Jewish Center for Immunology and Respiratory Disease, Denver, CO). A truncated form of SEB lacking the leader sequence, which spans base pairs 46 to 773 (referred to herein as SEB.S), was prepared by PCR amplification using the primers 5' GGGAATTCCATGGAGAGTCAACCAG 3' (SEQ ID NO:7) and 3' GCGGATCCTCACTTTTTCTTTGT 5' (SEQ ID NO:8). A truncated form of SEA lacking the signal sequence, which spans base pairs 46 to 751 (referred to herein as SEA.S), was prepared by PCR amplification using the primers 5' GGGAATTCCATGGAGAGTCAACCAG 3' (SEQ ID NO:9) and 5' GCAAGCTTAACTTGTATATAAATAG 3'(SEQ ID NO:10). Full-length cDNA encoding Toxic Shock Syndrome Toxin (TSST; SEQ ID NO:5) was produced by PCR amplification using a template obtained from Dr. Brian Kotzin (National Jewish Center for Immunology and Respiratory Disease, Denver, CO), using the primers:

5' CGGGGTACCCCGAAGGAGGAAAAAAAAATGTCTACAAACGATAATATAAAG 3' (SEQ ID NO:11); and

3' TGCTCTAGAGCATTAATTAATTTCTGCTTCTATAGTTTTTAT 5' (SEQ ID NO:12).

Each cDNA clone was ligated into the eukaryotic expression vector PCR_3 (In Vitrogen, San Diego, CA) using standard cloning methods. The full-length SEB cDNA cloned into PCR_3 is referred to herein as PCR_3-SEB; the full-length SEA cDNA cloned into PCR_3 is referred to herein as PCR_3-SEA;

the full-length TSST cDNA cloned into PCR_3 is referred to herein as PCR_3-TSST; the truncated SEB cDNA cloned into PCR_3 is referred to herein as PCR_3-SEB.S; and the truncated SEA cDNA cloned into PCR_3 is referred to herein as PCR_3-SEA.S.

A cDNA for canine GM-CSF was produced by PCR amplification of total RNA extracted from Concavalin A-stimulated normal canine peripheral blood mononuclear cells (PBMC) using canine GM-CSF primers designed based on the published canine GM-CSF cDNA (Nash, *ibid.*). The total RNA was reverse transcribed using the reverse transcriptase enzyme and oligoT primers. The canine GM-CSF cDNA was then amplified using PCR and specific 5′ and 3′ primers. The PCR product was cloned into the PCR_3 vector, the resulting recombinant molecule is referred to herein as PCR_3-GM_3.

Example 2

This example describes the expression of DNA encoding superantigens in mammalian CHO cells following transfection.

Isolated PCR_3-SEB.S, PCR_3-SEA.S and PCR_3-TSST were transformed into *E. coli* cells and ampicillin-resistant bacterial colonies were screened for the presence of the plasmid. Selected colonies were then cultured in large scale culture (liter volume). Plasmid DNA was isolated using standard methods. A typical plasmid yield was 20 mg plasmid DNA from one liter of bacteria-containing culture medium. Plasmid DNA was transfected into Chinese hamster ovary cells (CHO) by lipofection (Lipofectamine, Gibco-BRL, Gaithersburg, MD) using methods provided by the

manufacturer. About 2.0 μg of each plasmid DNA was separately transfected into about 10^6 CHO cells.

The transfected CHO cells were cultured for 48 hours. Supernatants and cell lysates were then isolated to determine the amount of intracellular and secreted SAg protein produced by the transfected cells. Cell lysates were prepared by detaching and sonicating the transfected cells to prepare cell lysates to measure activity. SAg protein activity in each sample was measured by quantitating the ability of the SAg protein to stimulate lymphocyte contained in a PBMC population using the following method. Supernatants and lysates to be tested were added in serial dilutions to triplicate wells of a 96-well plate containing 5 X 10^5 PBMC in a total volume of 200 μl per well. After 3 days, the wells were pulsed with ^3H thymidine and incubated for 18 hours. The radioactivity incorporated into the PBMC's were quantitated on a beta counter. Negative controls included CHO cells transfected with the DNA vector without an inserted gene (mock) and positive controls were purified recombinant SAg proteins.

The results were plotted as the mean incorporated thymidine in counts per minute and are shown in Fig. 1. The results indicate that both supernatants and lysates of CHO cells transfected with PCR_3-SEB.S, PCR_3-SEA.S and PCR_3-TSST stimulated strong proliferation of the PBMC's, compared to mock transfected cultures. The activity in supernatants in some cases exceeded that in cell lysates.

Thus, DNA encoding bacterial SAg proteins are capable of being transcribed and translated in mammalian cells in biologically active form. The results also indicate that the amounts of biologically active SAg protein are active produced by the transfected cells was sufficient to stimulate T cell proliferation.

Example 3

This example describes the expression of DNA encoding superantigens in canine melanoma cells following transfection.

A melanoma cell line was established from an oral malignant melanoma obtained by biopsy from a canine patient by isolating a portion of a melanoma tumor, digesting that portion with collagenase and plating the released cells in 24 well plates using Iscove Modified Dulbecco's Medium (IMDM) with 10% fetal calf serum. Melanoma cells were transfected with PCR_3-SEB.S, PCR_3-SEA.S and PCR_3-TSST by lipofection as described in Example 2. The cells were then irradiated (15,000 Rads). Four samples of each sample of transfected melanoma cells were prepared, in which decreasing numbers of the transfected cells were added to normal canine PBMC (5×10^5/well). Each sample was prepared in triplicate in a 96 well plate. After 3 days, proliferation was quantitated as described in Example 2. Non-transfected melanoma cells were used as negative control samples. The results were plotted as the mean incorporated thymidine in counts per minute and are shown in Fig. 2. The results indicate that Canine PBMC

proliferated when cultured with canine melanoma cells transfected with PCR_3-SEB.S, PCR_3-SEA.S and PCR_3-TSST, exhibiting a dose-dependent increase in proliferation as increasing numbers of irradiated tumor cells were used. Thus, melanoma tumor cells can be transfected and can express biologically active SAg protein. The results also show that the transfected melanoma cells continue to release biologically active SAg protein after irradiation, indicating that transfected tumor cells would also be useful as an autologous tumor vaccine as described in detail in the present application.

Example 4

This example describes the long term expression of DNA encoding SEB.S and SEA.S in stably transfected CHO cells.

To determine whether the SAg protein activity detected in supernatants of transfected CHO cells (described in Example 2) represented actual secretion or simple release from dying cells, stably transfected CHO lines were prepared using either PCR_3-SEB.S, PCR_3-SEA.S or vector with no cDNA insert (control). About 2 x 10^6 CHO cells were transfected with about 2 μg of plasmid DNA by lipofection. The transfected cells were then cultured in G418 (1 mg/ml) for 3 weeks to obtain stable transfectants. The G418 selected CHO cells were seeded into 9 individual tissue culture wells, allowed to adhere for 4 hours, and then fresh tissue culture media was added. Supernatants were harvested sequentially, beginning at time zero and continuing for 36 hours. Supernatants were added to PBMC

to assay for SAg protein activity, as described in Example 2.

The results were plotted as the mean proliferation stimulating activity contained in supernatants at each time point and are shown in Figs. 3A and 3B. The results indicate that a steady time-dependent increase in PBMC stimulatory activity was observed in supernatants from CHO cells stably transfected with both PCR_3-SEB.S and PCR_3-SEA.S. Thus, transfection of mammalian cells with PCR_3-SEB.S, PCR_3-SEA.S results in long term expression of biologically active SAg protein. The data indicates that transfected mammalian cells can serve as a sustained source of SAg protein production.

Example 5

This example describes that transfection of PCR_3-SEA.S DNA in melanoma cells results in the expression of biologically active SEA.S protein.

Superantigens are capable of stimulating the proliferation of T cells bearing certain Vβ domains in their T cell receptor (TCR). SEA is known to stimulate T cells having a Vβ3+ TCR in mice. SEB does not stimulate Vβ3+ T cells. Therefore, an experiment was performed to assess the ability of SEA.S protein expressed by melanoma cells transfected with PCR_3-SEA.S DNA to stimulate the proliferation of a T cell clone (AD10) expressing the Vβ3+TCR.

B16 melanoma cells were transfected with PCR_3-SEA.S DNA, PCR_3-SEB.S or PCR_3 vector DNA with no insert (mock).

The cells were then irradiated (18,000 Rads) and plated in triplicate in a 96 well plate at a concentration of about 1 x 10^4 per well. About 1 x 10^5 AD10 cells were added to each well. Next, irradiated syngeneic spleen cells were added to each well as a source of antigen presenting cells for the superantigen and T cell interaction. Negative controls included mock transfected cells; positive controls included recombinant SEA (10ng/ml). The cells were incubated for 48 hours. ^3H thymidine was then added to each well and the proliferative response quantitated.

The results were plotted as the mean incorporated thymidine in counts per minute and are shown in Fig. 4. The AD10 cells proliferated strongly in response to SEA.S protein produced by the PCR_3-SEA.S DNA transfected into the B16 cells, with the proliferative response nearly equal to that of the recombinant protein. Thus, the T cell response generated by transfection of melanoma cells with PCR_3-SEA.S DNA is specific for the correct TCR. Cells transfected with PCR_3-SEB.S DNA did not stimulate proliferation of AD10 cells, consistent with the predicted TCR specificity of SEA and SEB.

Example 6

This example describes the expression of PCR_3-GM DNA in CHO cells.

PCR_3-GM DNA was produced, isolated and transfected into CHO cells using the methods described in Examples 1 and 2. Expression of GM-CSF protein in the CHO cells was measured by the following method. Supernatants were isolated from

the cultures of the transfected cells and non-transfected CHO cells. The supernatants were added to cultures of monocyte cells (obtained from normal canine PBMC) and the ability of the supernatants to support the growth and survival of monocytes was determined. After 4 days in culture with test or control CHO supernatants, monocyte survival was quantitated by addition of methyltetrazolium dye (MTT) that is reduced in viable cells. Absorbance of light at 570 nm (measured using an ELISA reader) is representative of cell survival.

The results are shown in Fig. 5 and indicate that the supernatants from CHO transfected with PCR_3-GM DNA stimulated the survival of canine monocytes in culture compared with results obtained using the control supernatants. The level of activity was comparable to that of 1 x 10^5 units of canine recombinant GM-CSF. Thus, the GM-CSF protein produced by CHO cells transfected with PCR_3-GM DNA is biologically active.

Example 7

This example demonstrates that the vaccination of mice with autologous tumor cells transfected with PCR_3-SEA.S DNA or PCR_3-SEB.S DNA induce strong cytotoxic T cell (CTL) activity.

The following experiment studies the ability of non-immunogenic murine melanoma cells (B16 melanoma cells, F10 clone) expressing either PCR_3-SEA.S DNA or PCR_3-SEB.S to induce CTL responses in mice. B16 cells are known to be non-immunogenic when injected into C57B16/J mice. The

level of CTL responses that can be induced has been shown to correlate with the ability of the immunized animal to reject tumors.

B16 cells were transfected with either PCR_3-SEA.S DNA, PCR_3-SEB.S or PCR_3 DNA lacking insert (mock) using the method described in Example 2. The cells were then irradiated at 12,000 Rads. About 10^6 irradiated cells were then injected subcutaneously into C57B16/J mice. Three weeks later, the mice were sacrificed and their spleen mononuclear cells harvested. Mononuclear cells isolated from the spleen cells were then restimulated *in vitro* with irradiated, non-transfected wild type B16 cells for 6 days in media with interleukin-2 (IL-2). The spleen cells were then added in decreasing numbers to about 5×10^3 of ^{51}Cr-labeled wild type (non-transfected) B16 cells in a standard chromium release assay for CTL activity. After 4 hours, the supernatants were harvested and the percentage of specific lysis of the target B16 melanoma cells was quantitated.

The results are shown in Figs. 6A and 6B and indicate that injection of animals with irradiated transfected melanoma cells induce greater CTL activity than injection with non-transfected cells. This result is consistent with the non-immunogenic nature of B16 cells. Thus, DNA encoding bacterial SAg proteins expressed in transfected tumor cells are capable of eliciting strong CTL-mediated immunity against the non-transfected parental cell. These results suggest that autologous tumor cells transfected

with DNA encoding a superantigen constitute an effective tumor vaccine for treatment or prevention of metastatic disease.

Example 8

This example demonstrates that tumor cells transfected with PCR_3-SEB.S DNA are capable of inducing cytotoxic activity in adjacent T cells.

T cells were prepared from a mouse immunized with non-transfected B16 cells using the methods described in Example 7. These isolated cells exhibited minimal CTL activity towards non-transfected B16 target cells. B16 cells were transfected with PCR_3-SEB.S using the methods generally described in Example 2. Induction of CTL activity by the transfected B16 target cells was assessed in a standard 4 hour chromium release assay as used in Example 7.

The results are shown in Fig. 7 and indicates that B16 cells transfected with PCR_3-SEB.S produced protein that rapidly induced a four-fold increase in CTL activity in T cells that were relatively unresponsive to non-transfected target B16 cells. Thus, the SEB produced in the vicinity of the isolated T cells by the B16 cells is capable of stimulating such T cells. The data indicates that tumor cells transfected *in vivo* with PCR_3-SEB.S can produce biologically active SEB.S that is capable of rapidly activating T lymphocytes in their vicinity and thereby inducing cytotoxic activity against themselves or neighboring tumor cells.

Example 9

This example describes the treatment of canine melanoma with DNA encoding superantigen or GM-CSF.

A. Criteria for entry and trial design

Animals selected for entry into the present study were client owned animals with spontaneous oral malignant melanoma, a highly malignant neoplasm of dogs for which there is no alternative effective treatment. Prior to entry, the owners were required to sign informed consent. The study consisted of an initial 12 week trial response phase with 6 injections given once every 2 weeks, followed by long term once monthly maintenance therapy for those animals that responded during the initial 12 week induction phase. Potential toxicity was assessed by (1) body temperature measured daily for 7 days after injection; (2) physical examination of the injection site; (3) owner's assessment of their pet's attitude and appetite; (4) complete blood counts and biochemistry measurements once monthly. Treatment responses were assessed by: (1) physical measurement of tumor dimensions; (2) tumor photography; (3) thoracic radiographs for metastasis evaluation.

B. Superantigen + GM-CSF Treatment protocol

DNA samples complexed with liposomes were prepared as follows. PCR_3-SEB.S and PCR_3-GM plasmid DNA prepared from bacterial cultures by the alkaline lysis method, then purified by CsCl banding, were resuspended at a 1.0 mg/ml concentration in sterile PBS. Liposomes were prepared by

mixing equimolar amounts of N-[1-(33-dioleyloxy)propyl]-N,N,N-triethylammonium (DOTMA; obtained from Syntex, Corp., Palo Alto, CA) and dioleoyl phosphatidylethanolamine (DOPE; obtained from Avanti Polar Lipids, Birmingham, AL). The lipids were dried in a desiccator and reconstituted at a concentration of 1.0 mg/ml in sterile phosphate buffered saline (PBS), pH 7.0. The reconstituted lipids were sonicated for 5 minutes to produce liposomes having an average size of about 200 nm to about 400 nm.

Thirty minutes prior to injection into the patients, the PCR_3-SEB.S and PCR_3-GM DNA was mixed with the liposomes at a ratio of 1.0 μg DNA to 4 nmol liposome, in 1.0 ml sterile PBS. The solution was allowed to complex at room temperature. Two doses of DNA were administered, depending on tumor volume. For tumors less than 3 centimeters (cm) in diameter, 400 μg total DNA (200 μg each of PCR_3-SEB.S and PCR_3-GM DNA) were injected into each tumor. For tumors larger than 3 cm diameter, a total of 800 μg DNA (400 μg each of PCR_3-SEB.S and PCR_3-GM DNA) were injected into each tumor.

For each treatment, the DNA:liposome mixture was injected into the tumor site with a 3 ml syringe and 25 gauge needle. For larger tumors, most of the injection was delivered into tissues at the periphery of the tumor base. For some smaller tumors, injections were also injected directly into tumor tissue. Lymph node tissue having evidence of tumor metastasis was also injected. Injections were performed once every 2 weeks for the first 12 weeks,

then continued twice monthly for those animals in which an initial treatment response occurred, until complete tumor regression occurred. At that time, the frequency of injections decreased to once monthly. The toxicity of the treatment was evaluated based on the parameters outlined above in section A. The results are shown below in Table 1.

Table 1. Patient Log for SEB.S and PCR_3-GM DNA Treatment of Canine Melanoma

Patient	Stage	TN	Tumor Size	Start Date	Response	Comments
Zomax	I	T1bNOMO	1.5 cm diam	5/16/94	CR 51 wks	SEB.S + GM-CSF
Shadow	III	T2bN1bMO	3 cm diam	5/23/94	CR 50 wks	SEB.S + GM-CSF
NG	I	T1NOMO	1.2 cm diam	9/12/94	CR 34 wks	SEB.S + GM-CSF
Maggie	II	T2aNOMO	2 cm diam	8/24/94	PR 33 wks	SEB.S + GM-CSF
K.C.	III	T3aNOMO	> 4 cm diam	10/13/94	SD 12 wk	SEB.S + GM-CSF
Belvedere	III	T2N1bMO	4 cm diam	10/13/94	CR 30 wks	SEB.S + GM-CSF
Nicholas	III	T3bNOMO	> 4 cm diam	2/15/95	SD 12 wks	SEB.S + GM-CSF
Heidi	III	TON1bMO	LN:2cm diam	2/27/95	PR 10 wks	SEB.S + GM-CSF
Bear	III	TON1bMO	LN:2.5cm	4/11/95	SD 4 wks	SEB.S + GM-CSF

K y to terminology in patient data sheets:

Stage: I represents the smallest and III the largest size, with metastases
TNM: World Health Organization staging system
SD = stable disease (no tumor growth)
PR = partial remission (> 50% decrease in tumor size)
CR = tumor completely regressed
PD = progressive disease, no response to treatment
MCT = mast cell tumor
Mammary CA = mammary gland adenocarcinoma (malignant breast cancer)
Thyroid CA = thyroid adenocarcinoma
SCC = squamous cell carcinoma

The results shown in Table 1 indicate that a treatment response was observed in 6 of 9 dogs treated for the 12 week trial period. This included 4 complete remissions (no residual tumor) and 2 partial remissions (greater than 50% reduction in tumor size). Tumors in the remaining two dogs did not regress, but also did not progress in size during the 12 week trial. On average, a tumor response required 6 to 10 weeks to become apparent. The injections did not cause any inflammation or necrosis at injections sites. Toxicity, either local or systemic, was not observed in any of the 10 patents treated in this study. These results provide evidence of the efficacy of direct DNA injection using DNA encoding superantigen (SEB) and cytokine (GM-CSF) for treatment of spontaneous malignant melanoma in an outbred species.

Canine melanoma is a highly malignant, rapidly growing tumor of dogs, and provides a useful model for the study of treatments for human melanoma. Without treatment, the 50% survival time for animals with stage III disease (5 of the patients in this study) is about 3 months and all animals will be dead by 5 months due to pulmonary metastases. The observation of several long term survivors shown in Table 1 (others have not been treated long enough to evaluate) suggests that the combined DNA immunotherapy approach also has a systemic effect on preventing metastatic diseases.

Another major advantage of this approach is the apparent complete absence of toxicity in the dogs. Since dogs respond to SAg protein similar to humans, it is also

likely that toxicity in humans would also be minimal. The delivery of DNA encoding superantigens into tumor cells by transfection and subsequent local expression is sufficient to induce a strong immune response without inducing toxicity. Thus this genetic approach to tumor immunotherapy offers advantages over conventional chemotherapy and radiation therapy in terms of reducing patient morbidity. In addition, delivering the SAg protein by DNA transfection also avoids the potential toxicity associated with systemic administration.

C. Single Gene Treatment Protocol

To evaluate the effectiveness of injecting DNA encoding either a superantigen or a cytokine, relative to combined genetic therapy (SAg-encoding DNA and cytokine-encoding DNA), 2 groups of dogs were treated with either PCR_3-SEB.S DNA alone (3 dogs) or PCR_3-GM DNA alone (3 dogs; 2 entered, one evaluatable). Similar criteria for entry and trial design described above in Section A of this example was applied. Although not formally randomized, after the first 10 dogs were treated with the 2 gene combination, the next 3 enrollees were assigned the PCR_3-SEB.S DNA alone group and the next 3 to the PCR_3-GM DNA alone group. A similar treatment protocol as described above in section B was applied in this study. Briefly, the DNA was complexed with liposomes and injected once every 2 weeks for the first 12 weeks, then continued twice monthly for those animals in which an initial treatment response occurred, until complete tumor regression occurred. The

toxicity of the treatment was evaluated based on the parameters outlined above in section A. The results are shown below in Table 2.

Table 2. Patient Log for SEB.S or PCR_3-GM DNA alone Treatment of Canine Melanoma

Patient	Stage	TN	Tumor Size	Start Date	Response	Comments
Jessie	II	T2bNOMO	2 cm diam	1/11/95	PD 17 wks	SEB.S alone
Mr. T	III	TON1bMO	LN:2cm diam	2/1/95	PD 14 wks	SEB.S alone
Duffy	II	T2aNOMO	2 cm diam	2/3/95	PD 12 wks	SEB.S alone
Scooter	I	T2aNOMO	2 cm diam	3/24/95	PD 7 wks	GM-CSF alone

The results indicated that a tumor response did not occur in any dog receiving PCR_3-SEB.S DNA alone and tumors grew progressively. In addition, one dog (Scooter) treated with PCR_3-GM DNA alone also exhibited progressive growth. These data indicate that treatment with PCR_3-SEB.S DNA alone or PCR_3-GM DNA alone does not induce tumor regression. The data indicate that the marked anti-tumor efficacy of direct DNA injection results from the combined expression of PCR_3-SEB.S DNA and PCR_3-GM DNA in a tumor and adjacent tissues.

Example 10

This example describes the treatment of various tumor types with superantigen or GM-CSF encoding DNA.

The efficacy and lack of toxicity of PCR_3-SEB.S DNA and PCR_3-GM DNA was determined for the treatment of dogs with malignancies having similar biological and histological characteristics as human cancers. Dogs with five different cancers (advanced mammary carcinoma, mast cell tumor, thyroid carcinoma, non-oral melanoma, and squamous cell carcinoma) were treated in this study. Animals selected for entry into the present study included dogs with spontaneous malignancies that had received alternative treatments (e.g., chemotherapy and/or surgery) and either, had not responded, or had relapsed.

Therapeutic samples were prepared and injected intratumorally with PCR_3-SEB.S DNA and PCR_3-GM DNA as described above in Example 2. The dogs were treated initially once every 2 weeks for 12 weeks, then continued twice monthly for those animals in which an initial

treatment response occurred. The toxicity of the treatment was evaluated based on the parameters outlined above in Example 9, section A. The results are shown below in Table 3.

Table 3. Patient Log for SEB.S and PCR_3-GM DNA Treatment of Various Carcinomas

Patient	Tumor Type	Stage	TN	Tumor Size	Start Date	Response	Comments
Emma	Mammary CA	III	T4N1bNMO	1.8 cm diam	8/11/94	PR 22 wks	SEB.S + GM-CSF
Baby	Mammary CA	II	T1aN1bMO	2.6 cm diam	9/12/94	PR 8 wks	SEB.S + GM-CSF
Christa	MCT	IIIa	NA	>2 cm diam	7/27/94	SD 39 wks	SEB.S + GM-CSF
Jack	MCT	IIIa	NA	>3 cm diam	3/28/95	PD 4 wks	SEB.S + GM-CSF
Britt	Thyroid CA	III	T3bNOMO	>7 cm diam	10/14/94	SD 16 wk	SEB.S + GM-CSF
Duncan	Melanoma Toe	NA*	T2N1MO	>4 cm diam	8/11/94	SD 20 wks	SEB.S + GM-CSF
Billy	Melanoma Toe	NA*	TON1bMO	LN 3.5 cm	1/10/95	CR 17 wks	SEB.S + GM-CSF
Scotche	SCC Tonsil	NA	T3NOMO	4 cm diam	3/27/95	SD	SEB.S + GM-CSF

* Metastases
NA Not Applicable
CA Carcinoma
MCT Mast Cell Tumor
SCC Squamous Cell Carcinoma

In this study, toxicity was not observed in any of the animals. Tumor responses (partial remission of the primary tumors) were observed in the animals with mammary carcinoma and neither animal developed additional metastatic disease during the course of the study. Treatment of one dog (Billy) with a large, metastatic (lymph node metastases), non-oral melanoma resulted in complete remission of the cancer. Treatment of the other dog (Duncan) with a large, metastatic (lymph node metastases), non-oral melanoma resulted in prolonged stabilization of the disease. The dog with thyroid cancer (Britt) also experienced prolonged stabilization of the disease with once monthly injections. The response rate for the dogs with mast cell tumors was low. The effectiveness of the treatment on the squamous cell carcinoma is in early stages of evaluation. Taken together, the results indicate that PCR_3-SEB.S DNA and PCR_3-GM DNA can effectively treat multiple tumor types, in addition to the melanomas reported above in Example 9.

Example 11

This example describes the injection of PCR_3-SEA.S DNA into muscle cells which induces potent, long-lasting T cell deletion.

Four groups of mice B10.BR (2-3 mice per group) were prepared as follows. Group (1) consisted of untreated mice (control mice). Group (2) consisted of mice injected intraperitoneally with 100 ng of recombinant SEA (rSEA) protein. Group (3) consisted of mice injected

intramuscularly with 100 μg of PCR_3-SEA.S DNA (50 μg per leg, total of 100 μg/mouse). Group (4) consisted of mice injected intramuscularly with 100 μg PCR_3 (no insert; mock) DNA (50 μg per leg, total of 100 μg/mouse). The DNA samples were prepared by diluting 100 μl of a solution containing 100 μg of DNA 50:50 (v:v) in sterile PBS prior to injection. The rSEA protein was purified from cultures of *E. coli* cells transformed with the recombinant molecule PKK223 (obtained from Dr. John Kappler) encoding the truncated SEA.S protein lacking a leader sequence.

Beginning 72 hours after injection, mice were tail bled and PBMC prepared for fluorescence activated cell sorter (FACS) analysis. Cells were double labeled with the monoclonal antibodies FITC conjugated-GK1.5 antibody, biotinylated-KJ25 antibody and biotinylated-F23.1, to analyze for expression of CD4, TCR Vβ3 and TCR Vβ8 expression, respectively. The labelled cells were analyzed on an EPICS-C flow cytometer.

The percentage of cells isolated from the experimental mice expressing CD4 that also expressed either Vβ8 or Vβ3 was calculated and compared to percentages expressed by cells isolated from control mice. The mean percentage of CD4+ and Vβ3+ T cells in PBMC was plotted against time after injection. The results are shown in Fig. 8 and indicate that the percentage of CD4+, Vβ3+ T cells declined rapidly in PBMC of mice that received intramuscular injections with PCR_3-SEA.S DNA, but not in mice mock

injected with mock DNA. The percentages of Vβ8+ cells was not affected. This result is predicted since SEA protein does not bind mouse Vβ8+ T cells. The decline of the percentage of CD4+, Vβ3+ T cells occurred as rapidly as in mice injected with the recombinant SEA protein (rSEA). The deletion, however, observed over the next 2 months in mice injected with PCR_3-SEA.S DNA was longer lasting and was more pronounced than the deletion induced by injection of SEA.S protein. In addition, injection of as little as 2 μg PCR_3-SEA.S DNA also induced deletion of Vβ3+ T cells. Thus, intramuscular injection of DNA encoding superantigens represents a potent and non-toxic approach to the deletion or suppression of potentially harmful (e.g., autoreactive T cells) T cells.

Example 12

This example describes the production of immunogen and chemokine encoding recombinant molecules.

Recombinant molecules encoding ovalbumin (OVA) were produced by ligating cDNA encoding OVA into the eukaryotic expression vector PCR_3 and is referred to herein as PCR_3-OVA. cDNA encoding murine RANTES, murine macrophage inflammatory protein-1 alpha (MIP-1α), and macrophage inflammatory protein-1 beta (MIP-1β) was prepared from RNA isolated from LPS-stimulated normal murine bone marrow macrophages using methods standard in the art. The cDNA were ligated into the expression vector PCR_3, and are referred to herein as PCR_3-RANTES, PCR_3-MIP-1α and PCR_3-MIP-

1β. All plasmid DNA were purified by cesium chloride gradient centrifugation and resuspended at 1.0 mg/ml in sterile PBS.

Example 13

This example demonstrates that the co-administration of adjuvant DNA and immunogen DNA stimulates antibody production against the immunogen protein.

Separate groups of 4 CB6 F1 mice per group were injected twice with the following mixtures of DNA: (1) about 100 μg PCR_3-OVA + about 100 μg PCR_3-MIP-1β; (2) about 100 μg PCR_3-OVA + about 50 μg PCR_3-SEB (described in Example 1) + PCR_3-GM-CSF (described in Example 1); (3) about 100 μg PCR_3-OVA + about 100 μg PCR_3-RANTES; (4) about 100 μg PCR_3-OVA + about 100 μg PCR_3-SEB; (5) about 100 μg PCR_3-OVA + about 100 μg PCR_3-GM-CSF; or (6) about 100 μg PCR_3-OVA alone. Control samples were also prepared which included 6 non-injected, syngeneic mice. The DNA was diluted to a final concentration of 0.5 mg/ml in sterile phosphate buffered saline (PBS) prior to injection. The mice were injected intramuscularly, bilaterally in their quadriceps muscles (about 100 μg of DNA per quadricep).

About 20 days after the immunization of step B, serum was collected from each mouse and assayed for antibodies that specifically bind to OVA protein using an OVA-specific enzyme linked immunoassay (ELISA) assay using methods standard in the art. Briefly, OVA protein was bound to an ELISA plate. The plates were washed and then incubated in

the presence of serum. Again the plates were washed and then incubated in the presence of HRP-conjugated anti-mouse IgG antibody. The amount of antibody bound to the OVA was detected on an ELISA reader and are expressed in absorbance units.

The results of the ELISA are shown in Fig. 9 and indicate that co-injection of DNA encoding OVA, with either DNA encoding RANTES or MIP-1β, or SEB and GM-CSF, increases the antibody response to OVA over that observed with OVA alone, OVA plus GM-CSF, OVA plus SEB alone or control samples. Thus, the expression of RANTES, MIP-1β, or SEB and GM-CSF increase the antibody response to OVA when administered as a DNA vaccine.

Example 14

This example demonstrates that the co-administration of DNA adjuvant and immunogen DNA results in the production of interferon gamma.

Separate groups of 4 CB6 F1 mice per group were injected twice, intramuscularly (on day 1 and day 21), with the following mixtures of DNA: (1) about 100 μg PCR_3-OVA + about 100 μg PCR_3-MIP-1β; (2) about 100 μg PCR_3-OVA + about 50 μg PCR_3-SEB + PCR_3-GM-CSF; (3) about 100 μg PCR_3-OVA + about 100 μg PCR_3-RANTES; (4) about 100 μg PCR_3-OVA + about 100 μg PCR_3-SEB; (5) about 100 μg PCR_3-OVA + about 100 μg PCR_3-GM-CSF; or (6) about 100 μg PCR_3-OVA alone. Control samples were also prepared as above.

The mice were sacrificed on day 27. Spleen cells were harvested from each mouse and re-stimulated *in vitro* with irradiated OVA-transfected cells (EG7-OVA) in quadruplicate wells. On day 4 of the re-stimulation with irradiated EG7-OVA cells, supernatants were harvested from the cultures and assayed for interferon gamma activity using an interferon gamma-specific ELISA assay. Results were expressed as units/ml of interferon activity, as determined by comparison with a standard curve generated with recombinant murine interferon-gamma.

The results are shown in Fig. 10 and indicate that RANTES or GM-CSF were effective compounds for inducing interferon-gamma production. Although less, SEB and MIP-1β also induced interferon-gamma production. Additional experiments indicated that none of the adjuvants evaluated in this experiment induced significant quantities of IL-4 release. Together, these data indicate that the immune response induced by an adjuvant of the present invention is primarily a Th1 response, which induces primarily cell-mediated immunity, including macrophage activation, enhanced T cell CTL activity, and increased MHC expression.

Example 15

This example demonstrates that the co-administration of adjuvant DNA and immunogen DNA induce T cell proliferative responses to the immunogen.

Separate group of 4 CB6 F1 mice per group were immunized using the protocol described in Example 14. The

animals were sacrificed on day 27 and harvested spleen cells re-stimulated using the method described in Example 14. After about 4 days of re-stimulation, 100 μl aliquots of the cells were harvested from each well and pulsed for 18 hours with ^{3}H-thymidine. Thymidine incorporation was then quantitated (cpm) as a measure of the proliferative response to OVA expressed by the transfected EG7-OVA cell line.

The results are shown in Fig. 11 and indicate that MIP-1β, RANTES, SEB + GM-CSF, and SEB alone, when co-administered together with OVA DNA, induce a substantial increase in the proliferative response to OVA. Thus, these data provide evidence that DNA encoding chemokines and SAgs are useful for enhancing cell-mediated immune responses and therefore are useful as DNA vaccine adjuvants.

Example 16

This example demonstrates that the co-administration of adjuvant DNA increases CTL responses to the immunogen ovalbumin.

Mice were immunized using the protocol described in Example 14. Spleen cells were harvested from the immunized mice 7 days after the last vaccination. The cells were then re-stimulated *in vitro* for 6 days with irradiated EG7-OVA cells. T cells were then harvested from the re-stimulated population and added in decreasing numbers to ^{51}Cr-labeled EG7-OVA or EL-4 target cells in a standard 4 hour chromium release assay for CTL activity. The percent

cell lysis was determined Chromium release was then quantitated (cpm) as a measure of the percent specific cell lysis of labeled target cells. The higher the % specific lysis, the more CTL activity exhibited by the T cells.

The results are shown in Fig. 12 and indicate that all of the adjuvant DNAs evaluated induced increased CTL activity compared to OVA alone. The use of RANTES, GM-CSF and SEB alone, each were effective in inducing CTL activity. These data indicate that co-administration of chemokine DNA can enhance CTL-mediated immunity to an intracellular immunogen, as typified by OVA expressed in a transfected cell line, indicating that this approach is useful for vaccines against intracellular pathogens.

Taken together, the results of Examples 12-16 indicate that all DNA adjuvants tested (GM-CSF, SEB, SEB+GM-CSF, RANTES and MIP-1β) improved cell mediated immunity against the immunogen ovalbumin. In particular, the use of either SEB or GM-CSF alone, as well as the combination of SEB + GM-CSF were effective at inducing cell mediated immunity.

SEQUENCE LISTING

The following Sequence Listing is submitted pursuant to 37 CFR §1.821. A copy in computer readable form is also submitted herewith.

Applicants assert pursuant to 37 CFR §1.821(f) that the content of the paper and computer readable copies of SEQ ID NO:1 through SEQ ID NO:13 submitted herewith are the same.

(1) GENERAL INFORMATION:

(i) APPLICANT: Dow, Steve W.
Elmslie, Robyn E.
Potter, Terence A.

(ii) TITLE OF INVENTION: GENE THERAPY FOR EFFECTOR CELL REGULATION

(iii) NUMBER OF SEQUENCES: 13

(iv) CORRESPONDENCE ADDRESS:
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(F) ZIP: 80203

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Connell, Gary J.
(B) REGISTRATION NUMBER: 32,020
(C) REFERENCE/DOCKET NUMBER: 2879-29-C1-PCT

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 773 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..765

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GAG AGT CAA CCA GAT CCT AAA CCA GAT GAG TTG CAC AAA TCG AGT AAA      96
Glu Ser Gln Pro Asp Pro Lys Pro Asp Glu Leu His Lys Ser Ser Lys
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Phe Thr Gly Leu Met Glu Asn Met Lys Val Leu Tyr Asp Asp Asn His
        35                  40                  45

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Val Ser Ala Ile Asn Val Lys Ser Ile Asp Gln Phe Leu Tyr Phe Asp
    50                  55                  60

TTA ATA TAT TCT ATT AAG GAC ACT AAG TTA GGG AAT TAT GAT AAT GTT     240
Leu Ile Tyr Ser Ile Lys Asp Thr Lys Leu Gly Asn Tyr Asp Asn Val
65                  70                  75                  80

CGA GTC GAA TTT AAA AAC AAA GAT TTA GCT GAT AAA TAC AAA GAT AAA     288
Arg Val Glu Phe Lys Asn Lys Asp Leu Ala Asp Lys Tyr Lys Asp Lys
                85                  90                  95

TAC GTA GAT GTG TTT GGA GCT AAT TAT TAT TAT CAA TGT TAT TTT TCT     336
Tyr Val Asp Val Phe Gly Ala Asn Tyr Tyr Tyr Gln Cys Tyr Phe Ser
            100                 105                 110

AAA AAA ACG AAT GAT ATT AAT TCG CAT CAA ACT GAC AAA CGA AAA ACT     384
Lys Lys Thr Asn Asp Ile Asn Ser His Gln Thr Asp Lys Arg Lys Thr
        115                 120                 125

TGT ATG TAT GGT GGT GTA ACT GAG CAT AAT GGA AAC CAA TTA GAT AAA     432
Cys Met Tyr Gly Gly Val Thr Glu His Asn Gly Asn Gln Leu Asp Lys
    130                 135                 140

TAT AGA AGT ATT ACT GTT CGG GTA TTT GAA GAT GGT AAA AAT TTA TTA     480
Tyr Arg Ser Ile Thr Val Arg Val Phe Glu Asp Gly Lys Asn Leu Leu
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TCT TTT GAC GTA CAA ACT AAT AAG AAA AAG GTG ACT GCT CAA GAA TTA     528
Ser Phe Asp Val Gln Thr Asn Lys Lys Lys Val Thr Ala Gln Glu Leu
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Asp Tyr Leu Thr Arg His Tyr Leu Val Lys Asn Lys Lys Leu Tyr Glu
            180                 185                 190
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TTT AAC AAC TCG CCT TAT GAA ACG GGA TAT ATT AAA TTT ATA GAA AAT      624
Phe Asn Asn Ser Pro Tyr Glu Thr Gly Tyr Ile Lys Phe Ile Glu Asn
        195                 200                 205

GAG AAT AGC TTT TGG TAT GAC ATG ATG CCT GCA CCA GGA GAT AAA TTT      672
Glu Asn Ser Phe Trp Tyr Asp Met Met Pro Ala Pro Gly Asp Lys Phe
    210                 215                 220

GAC CAA TCT AAA TAT TTA ATG ATG TAC AAT GAC AAT AAA ATG GTT GAT      720
Asp Gln Ser Lys Tyr Leu Met Met Tyr Asn Asp Asn Lys Met Val Asp
225                 230                 235                 240

TCT AAA GAT GTG AAG ATT GAA GTT TAT CTT ACG ACA AAG AAA AAG          765
Ser Lys Asp Val Lys Ile Glu Val Tyr Leu Thr Thr Lys Lys Lys
                245                 250                 255

TGAAGCTT                                                            773
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 255 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Thr Met Ile Thr Asn Leu Ile Arg Leu Thr Ile Gly Asn Ser Met
  1               5                  10                  15

Glu Ser Gln Pro Asp Pro Lys Pro Asp Glu Leu His Lys Ser Ser Lys
            20                  25                  30

Phe Thr Gly Leu Met Glu Asn Met Lys Val Leu Tyr Asp Asp Asn His
        35                  40                  45

Val Ser Ala Ile Asn Val Lys Ser Ile Asp Gln Phe Leu Tyr Phe Asp
    50                  55                  60

Leu Ile Tyr Ser Ile Lys Asp Thr Lys Leu Gly Asn Tyr Asp Asn Val
65                  70                  75                  80

Arg Val Glu Phe Lys Asn Lys Asp Leu Ala Asp Lys Tyr Lys Asp Lys
                85                  90                  95

Tyr Val Asp Val Phe Gly Ala Asn Tyr Tyr Tyr Gln Cys Tyr Phe Ser
            100                 105                 110

Lys Lys Thr Asn Asp Ile Asn Ser His Gln Thr Asp Lys Arg Lys Thr
        115                 120                 125

Cys Met Tyr Gly Gly Val Thr Glu His Asn Gly Asn Gln Leu Asp Lys
    130                 135                 140

Tyr Arg Ser Ile Thr Val Arg Val Phe Glu Asp Gly Lys Asn Leu Leu
145                 150                 155                 160

Ser Phe Asp Val Gln Thr Asn Lys Lys Lys Val Thr Ala Gln Glu Leu
                165                 170                 175

Asp Tyr Leu Thr Arg His Tyr Leu Val Lys Asn Lys Lys Leu Tyr Glu
            180                 185                 190
```

```
Phe Asn Asn Ser Pro Tyr Glu Thr Gly Tyr Ile Lys Phe Ile Glu Asn
        195                 200                 205

Glu Asn Ser Phe Trp Tyr Asp Met Met Pro Ala Pro Gly Asp Lys Phe
    210                 215                 220

Asp Gln Ser Lys Tyr Leu Met Met Tyr Asn Asp Asn Lys Met Val Asp
225                 230                 235                 240

Ser Lys Asp Val Lys Ile Glu Val Tyr Leu Thr Thr Lys Lys Lys
                245                 250                 255
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 751 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 46..744

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
ATGACCATGA TTACGAATTT AATACGACTC ACTATAGGGA ATTCC ATG GAG AAA      54
                                                  Met Glu Lys
                                                    1

AGC GAA GAA ATA AAT GAG AAA GAT CTG CGC AAG AAG TCC GAA TTG CAG    102
Ser Glu Glu Ile Asn Glu Lys Asp Leu Arg Lys Lys Ser Glu Leu Gln
  5                 10                  15

GGA ACA GCC CTA GGC AAT CTT AAA CAA ATC TAT TAT TAC AAT GAA AAA    150
Gly Thr Ala Leu Gly Asn Leu Lys Gln Ile Tyr Tyr Tyr Asn Glu Lys
 20                  25                  30                  35

GCG AAG ACT GAG AAT AAA GAG AGT CAC GAT CAA TTT CTG CAG CAT ACT    198
Ala Lys Thr Glu Asn Lys Glu Ser His Asp Gln Phe Leu Gln His Thr
                40                  45                  50

ATA TTG TTT AAA GGC TTT TTT ACT GAT CAT TCG TGG TAT AAC GAT TTA    246
Ile Leu Phe Lys Gly Phe Phe Thr Asp His Ser Trp Tyr Asn Asp Leu
            55                  60                  65

CTA GTA GAT TTT GAT TCG AAG GAC ATC GTT GAT AAA TAT AAA GGG AAG    294
Leu Val Asp Phe Asp Ser Lys Asp Ile Val Asp Lys Tyr Lys Gly Lys
        70                  75                  80

AAG GTC GAC TTG TAT GGT GCT TAT TAT GGG TAC CAA TGT GCT GGT GGT    342
Lys Val Asp Leu Tyr Gly Ala Tyr Tyr Gly Tyr Gln Cys Ala Gly Gly
  85                  90                  95

ACA CCA AAC AAA ACA GCA TGC ATG TAT GGT GGG GTA ACC TTA CAT GAC    390
Thr Pro Asn Lys Thr Ala Cys Met Tyr Gly Gly Val Thr Leu His Asp
100                 105                 110                 115

AAT AAT CGA TTG ACC GAA GAG AAA AAG GTC CCG ATC AAT TTA TGG CTA    438
Asn Asn Arg Leu Thr Glu Glu Lys Lys Val Pro Ile Asn Leu Trp Leu
                120                 125                 130
```

```
GAC GGT AAA CAA AAT ACA GTA CCT CTA GAA ACG GTT AAA ACG AAT AAG      486
Asp Gly Lys Gln Asn Thr Val Pro Leu Glu Thr Val Lys Thr Asn Lys
          135                 140                 145

AAA AAT GTA ACT GTC CAA GAG CTG GAT CTT CAA GCG CGC CGA TAC CTA      534
Lys Asn Val Thr Val Gln Glu Leu Asp Leu Gln Ala Arg Arg Tyr Leu
      150                 155                 160

CAG GAA AAA TAT AAT TTG TAC AAC TCT GAC GTC TTT GAT GGG AAG GTT      582
Gln Glu Lys Tyr Asn Leu Tyr Asn Ser Asp Val Phe Asp Gly Lys Val
  165                 170                 175

CAG AGA GGC CTA ATC GTG TTT CAT ACT TCT ACA GAA CCT TCG GTT AAC      630
Gln Arg Gly Leu Ile Val Phe His Thr Ser Thr Glu Pro Ser Val Asn
180                 185                 190                 195

TAC GAT TTA TTT GGA GCT CAA GGA CAG TAT TCA AAT ACA CTC TTA AGA      678
Tyr Asp Leu Phe Gly Ala Gln Gly Gln Tyr Ser Asn Thr Leu Leu Arg
                200                 205                 210

ATA TAT CGC GAC AAC AAG ACG ATT AAC TCT GAA AAC ATG CAC ATT GAT      726
Ile Tyr Arg Asp Asn Lys Thr Ile Asn Ser Glu Asn Met His Ile Asp
          215                 220                 225

ATC TAT TTA TAT ACA AGT TAAGCTT                                     751
Ile Tyr Leu Tyr Thr Ser
      230
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 233 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Glu Lys Ser Glu Glu Ile Asn Glu Lys Asp Leu Arg Lys Lys Ser
 1               5                  10                  15

Glu Leu Gln Gly Thr Ala Leu Gly Asn Leu Lys Gln Ile Tyr Tyr Tyr
            20                  25                  30

Asn Glu Lys Ala Lys Thr Glu Asn Lys Glu Ser His Asp Gln Phe Leu
        35                  40                  45

Gln His Thr Ile Leu Phe Lys Gly Phe Phe Thr Asp His Ser Trp Tyr
    50                  55                  60

Asn Asp Leu Leu Val Asp Phe Asp Ser Lys Asp Ile Val Asp Lys Tyr
65                  70                  75                  80

Lys Gly Lys Lys Val Asp Leu Tyr Gly Ala Tyr Tyr Gly Tyr Gln Cys
                85                  90                  95

Ala Gly Gly Thr Pro Asn Lys Thr Ala Cys Met Tyr Gly Gly Val Thr
            100                 105                 110

Leu His Asp Asn Asn Arg Leu Thr Glu Glu Lys Lys Val Pro Ile Asn
        115                 120                 125

Leu Trp Leu Asp Gly Lys Gln Asn Thr Val Pro Leu Glu Thr Val Lys
    130                 135                 140
```

```
Thr Asn Lys Lys Asn Val Thr Val Gln Glu Leu Asp Leu Gln Ala Arg
145                 150                 155                 160

Arg Tyr Leu Gln Glu Lys Tyr Asn Leu Tyr Asn Ser Asp Val Phe Asp
                165                 170                 175

Gly Lys Val Gln Arg Gly Leu Ile Val Phe His Thr Ser Thr Glu Pro
            180                 185                 190

Ser Val Asn Tyr Asp Leu Phe Gly Ala Gln Gly Gln Tyr Ser Asn Thr
        195                 200                 205

Leu Leu Arg Ile Tyr Arg Asp Asn Lys Thr Ile Asn Ser Glu Asn Met
    210                 215                 220

His Ile Asp Ile Tyr Leu Tyr Thr Ser
225                 230
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 582 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..582

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
ATG ACA AAC GAT AAT ATA AAG GAT TTG CTA GAC TGG TAT AGT AGT GGG      48
Met Thr Asn Asp Asn Ile Lys Asp Leu Leu Asp Trp Tyr Ser Ser Gly
 1               5                  10                  15

TCT GAC ACT TTT ACA AAT AGT GAA GTT TTA GAT AAT TCC TTA GGA TCT      96
Ser Asp Thr Phe Thr Asn Ser Glu Val Leu Asp Asn Ser Leu Gly Ser
            20                  25                  30

ATG CGT ATA AAA AAC ACA GAT GGC AGC ATC AGC CTT ATA ATT TTT CCG     144
Met Arg Ile Lys Asn Thr Asp Gly Ser Ile Ser Leu Ile Ile Phe Pro
        35                  40                  45

AGT CCT TAT TAT AGC CCT GCT TTT ACA AAA GGG GAA AAA GTT GAC TTA     192
Ser Pro Tyr Tyr Ser Pro Ala Phe Thr Lys Gly Glu Lys Val Asp Leu
    50                  55                  60

AAC ACA AAA AGA ACT AAA AAA AGC CAA CAT ACT AGC GAA GGA ACT TAT     240
Asn Thr Lys Arg Thr Lys Lys Ser Gln His Thr Ser Glu Gly Thr Tyr
65                  70                  75                  80

ATC CAT TTC CAA ATA AGT GGC GTT ACA AAT ACT GAA AAA TTA CCT ACT     288
Ile His Phe Gln Ile Ser Gly Val Thr Asn Thr Glu Lys Leu Pro Thr
                85                  90                  95

CCA ATA GAA CTA CCT TTA AAA GTT AAG GTT CAT GGT AAA GAT AGC CCC     336
Pro Ile Glu Leu Pro Leu Lys Val Lys Val His Gly Lys Asp Ser Pro
            100                 105                 110

TTA AAG TAT TGG CCA AAG TTC GAT AAA AAA CAA TTA GCT ATA TCA ACT     384
Leu Lys Tyr Trp Pro Lys Phe Asp Lys Lys Gln Leu Ala Ile Ser Thr
        115                 120                 125
```

```
TTA GAC TTT GAA ATT CGT CAT CAG CTA ACT CAA ATA CAT GGA TTA TAT     432
Leu Asp Phe Glu Ile Arg His Gln Leu Thr Gln Ile His Gly Leu Tyr
    130                 135                 140

CGT TCA AGC GAT AAA ACG GGT GGT TAT TGG AAA ATA ACA ATG AAT GAC     480
Arg Ser Ser Asp Lys Thr Gly Gly Tyr Trp Lys Ile Thr Met Asn Asp
145                 150                 155                 160

GGA TCC ACA TAT CAA AGT GAT TTA TCT AAA AAG TTT GAA TAC AAT ACT     528
Gly Ser Thr Tyr Gln Ser Asp Leu Ser Lys Lys Phe Glu Tyr Asn Thr
                165                 170                 175

GAA AAA CCA CCT ATA AAT ATT GAT GAA ATA AAA ACT ATA GAA GCA GAA     576
Glu Lys Pro Pro Ile Asn Ile Asp Glu Ile Lys Thr Ile Glu Ala Glu
            180                 185                 190

ATT AAT                                                            582
Ile Asn
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 194 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Thr Asn Asp Asn Ile Lys Asp Leu Leu Asp Trp Tyr Ser Ser Gly
 1               5                  10                  15

Ser Asp Thr Phe Thr Asn Ser Glu Val Leu Asp Asn Ser Leu Gly Ser
            20                  25                  30

Met Arg Ile Lys Asn Thr Asp Gly Ser Ile Ser Leu Ile Ile Phe Pro
        35                  40                  45

Ser Pro Tyr Tyr Ser Pro Ala Phe Thr Lys Gly Glu Lys Val Asp Leu
    50                  55                  60

Asn Thr Lys Arg Thr Lys Lys Ser Gln His Thr Ser Glu Gly Thr Tyr
65                  70                  75                  80

Ile His Phe Gln Ile Ser Gly Val Thr Asn Thr Glu Lys Leu Pro Thr
                85                  90                  95

Pro Ile Glu Leu Pro Leu Lys Val Lys Val His Gly Lys Asp Ser Pro
            100                 105                 110

Leu Lys Tyr Trp Pro Lys Phe Asp Lys Lys Gln Leu Ala Ile Ser Thr
        115                 120                 125

Leu Asp Phe Glu Ile Arg His Gln Leu Thr Gln Ile His Gly Leu Tyr
    130                 135                 140

Arg Ser Ser Asp Lys Thr Gly Gly Tyr Trp Lys Ile Thr Met Asn Asp
145                 150                 155                 160

Gly Ser Thr Tyr Gln Ser Asp Leu Ser Lys Lys Phe Glu Tyr Asn Thr
                165                 170                 175

Glu Lys Pro Pro Ile Asn Ile Asp Glu Ile Lys Thr Ile Glu Ala Glu
            180                 185                 190

Ile Asn
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..25
(D) OTHER INFORMATION: /label= primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GGGAATTCCA TGGAGAGTCA ACCAG 25

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..23
(D) OTHER INFORMATION: /label= primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GCGGATCCTC ACTTTTTCTT TGT 23

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..22
(D) OTHER INFORMATION: /label= primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GGGAATTCCA TGGAGAAAAG CG 22

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..25
(D) OTHER INFORMATION: /label= primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GCAAGCTTAA CTTGTATATA AATAG 25

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..51

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CGGGGTACCC CGAAGGAGGA AAAAAAAATG TCTACAAACG ATAATATAAA G 51

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..42
(D) OTHER INFORMATION: /label= primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TGCTCTAGAG CATTAATTAA TTTCTGCTTC TATAGTTTTT AT 42

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 279 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ACCATGAAGA TCTCTGCAGC TGCCCTCACC ATCATCCTCA CTGCAGCCGC CCTCTGGGCG 60

CCCGCGCCTG CCTCACCATA TGGCTCGGAC ACCACTCCCT GCTGCTTTGC CTACCTCTCC 120

CTCGCGCTGC CTCGTGCCCA CGTCAAGGAG TATTTCTACA CCAGCAGCAA GTGCTCCAAT 180

CTTGCAGTCG TGTTTGTCAC TCGAAGGAAC CGCCAAGTGT GTGCCAACCC AGAGAAGAAG 240

TGGGTTCAAG AATACATCAA CTATTTGGAG ATGAGCTAG 279

While various embodiments of the present invention have been described in detail, it is apparent that modifications and adaptations of those embodiments will occur to those skilled in the art. It is to be expressly understood, however, that such modifications and adaptations are within the scope of the present invention, as set forth in the following claims:

What is claimed:

1. A therapeutic composition comprising: (a) an isolated nucleic acid molecule encoding a superantigen; and (b) an isolated nucleic acid molecule selected from the group consisting of a nucleic acid molecule encoding a cytokine, a nucleic acid molecule encoding a chemokine and mixtures thereof, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences.

2. The therapeutic composition of Claim 1, wherein said superantigen is selected from the group consisting of staphylococcal enterotoxins, retroviral antigens, streptococcal antigens, mycoplasma antigens, mycobacteria antigens, viral antigens and protozoan antigens.

3. The therapeutic composition of Claim 1, wherein said superantigen comprises staphylococcal enterotoxins.

4. The therapeutic composition of Claim 1, wherein said superantigen is selected from the group consisting of SEA, SEB, SEC_1, SEC_2, SEC_3, SED, SEE and TSST.

5. The therapeutic composition of Claim 1, wherein said superantigen is derived from a virus selected from the group consisting of mouse mammary tumor virus, rabies virus and herpes virus.

6. The therapeutic composition of Claim 1, wherein said cytokine is selected from the group consisting of hematopoietic growth factors, interleukins, interferons,

immunoglobulin superfamily molecules, tumor necrosis factor family molecules and chemokines.

7. The therapeutic composition of Claim 1, wherein said cytokine is selected from the group consisting of granulocyte macrophage colony stimulating factor, macrophage colony stimulating factor, tumor necrosis factor α, interleukin-1, interleukin-2, interleukin-4, interleukin-6, interleukin-12, and interleukin-15.

8. The therapeutic composition of Claim 1, wherein said cytokine is selected from the group consisting of granulocyte macrophage colony stimulating factor and tumor necrosis factor α.

9. The therapeutic composition of Claim 1, wherein said cytokine comprises granulocyte macrophage colony stimulating factor.

10. The therapeutic composition of Claim 1, wherein said chemokine is selected from the group consisting of C5a, IL-8, MIP1α, MIP1β, MCP-1, MCP-3, PAFR, FMLPR, LTB_4R, GRP, RANTES, eotaxin, lymphotactin, IP10, I-309, ENA78, GCP-2, NAP-2 and MGSA/gro.

11. The therapeutic composition of Claim 1, wherein said chemokine is selected from the group consisting of IL-8, MIP1α, MIP1β, MCP-1, MCP-3, RANTES and NAP-2.

12. The therapeutic composition of Claim 1, wherein said chemokine is selected from the group consisting of IL-8, Rantes, MIP1α and MIP1β.

13. The therapeutic composition of Claim 1, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences capable of being expressed in a mammalian cell.

14. The therapeutic composition of Claim 1, wherein said transcription control sequences are selected from the group consisting of RSV control sequences, CMV control sequences, retroviral LTR sequences, SV-40 control sequences and β-actin control sequences.

15. The therapeutic composition of Claim 1, wherein said isolated nucleic acid molecule encoding a superantigen comprises a recombinant molecule selected from the group consisting of PCR_3-SEB, PCR_3-SEA, PCR_3-SEB.S, PCR_3-SEA.S and PCR_3-TSST.

16. The therapeutic composition of Claim 1, wherein said isolated nucleic acid molecule encoding a cytokine comprises PCR_3-GM_3.

17. The therapeutic composition of Claim 1, wherein said isolated nucleic acid molecule encoding a chemokine is selected from the group consisting of PCR_3-RANTES, PCR_3-MIP1α and PCR_3-MIP1β.

18. The therapeutic composition of Claim 1, wherein said therapeutic composition further comprises a pharmaceutically acceptable carrier selected from the group consisting of an aqueous physiologically balanced solution, an artificial lipid-containing substrate, a natural lipid-

containing substrate, an oil, an ester, a glycol, a virus and a metal particle.

19. The therapeutic composition of Claim 18, wherein said pharmaceutically acceptable carrier is selected from the group consisting of liposomes and an aqueous physiologically balanced solution.

20. The therapeutic composition of Claim 1, wherein said therapeutic composition further comprises an immunogen selected from the group consisting of a pathogen, an allergen, tumor antigens and self-antigens.

21. The therapeutic composition of Claim 1, wherein said therapeutic composition further comprises an immunogen comprising a peptide derived from *Mycobacterium tuberculosis*.

22. A recombinant molecule comprising: (a) a first isolated nucleic acid molecule encoding a superantigen; and (b) a second isolated nucleic acid molecule selected from the group consisting of a nucleic acid molecule encoding a cytokine, a nucleic acid molecule encoding a chemokine, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences.

23. The molecule of Claim 22, wherein said recombinant molecule contains a transcription control sequence selected from the group consisting of RSV control sequences, CMV control sequences, retroviral LTR control

sequences, SV-40 control sequences and β-actin control sequences.

24. The molecule of Claim 22, wherein said recombinant molecule is dicistronic and comprises an IRES.

25. The molecule of Claim 22, wherein said first nucleic acid molecule and said second nucleic acid molecule are separated by an IRES.

26. The molecule of Claim 22, wherein said first nucleic acid molecule encodes a superantigen selected from the group consisting of SEA, SEB, SEC_1, SEC_2, SEC_3, SED, SEE and TSST.

27. The molecule of Claim 22, wherein said second nucleic acid molecule encodes a protein selected from the group consisting of macrophage colony stimulating factor, macrophage colony stimulating factor, tumor necrosis factor α, interleukin-1, interleukin-2, interleukin-4, interleukin-6, interleukin-12, interleukin-15, C5a, IL-8, MIP1α, MIP1β, MCP-1, MCP-3, PAFR, FMLPR, LTB_4R, GRP, RANTES, eotaxin, lymphotactin, IP10, I-309, ENA78, GCP-2, NAP-2 and MGSA/gro.

28. A recombinant molecule comprising: (a) a first isolated nucleic acid molecule encoding a first superantigen; and (b) a second isolated nucleic acid molecule encoding a second superantigen, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences.

29. The molecule of Claim 28, wherein said recombinant molecule contains a transcription control sequence selected from the group consisting of RSV control sequences, CMV control sequences, retroviral LTR control sequences, SV-40 control sequences and β-actin control sequences.

30. The molecule of Claim 28, wherein said recombinant molecule is dicistronic and comprises an IRES.

31. The molecule of Claim 28, wherein said first nucleic acid molecule and said second nucleic acid molecule are separated by an IRES.

32. The molecule of Claim 28, wherein said first nucleic acid molecule encodes a superantigen selected from the group consisting of SEA, SEB, SEC_1, SEC_2, SEC_3, SED, SEE and TSST.

33. A therapeutic composition comprising a delivery vehicle carrying: (a) a first isolated nucleic acid molecule encoding a superantigen; and (b) a second isolated nucleic acid molecule selected from the group consisting of a nucleic acid molecule encoding a cytokine, a nucleic acid molecule encoding a chemokine and mixtures thereof, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences.

34. The therapeutic composition of Claim 33, wherein said delivery vehicle comprises a liposome.

35. The therapeutic composition of Claim 33, wherein said first nucleic acid molecule encodes a superantigen

selected from the group consisting of SEA, SEB, SEC_1, SEC_2, SEC_3, SED, SEE and TSST.

36. The therapeutic composition of Claim 33, wherein said second nucleic acid molecule encodes a protein selected from the group consisting of macrophage colony stimulating factor, macrophage colony stimulating factor, tumor necrosis factor α, interleukin-1, interleukin-2, interleukin-4, interleukin-6, interleukin-12, interleukin-15, C5a, IL-8, MIP1α, MIP1β, MCP-1, MCP-3, PAFR, FMLPR, LTB_4R, GRP, RANTES, eotaxin, lymphotactin, IP10, I-309, ENA78, GCP-2, NAP-2 and MGSA/gro.

37. The therapeutic composition of Claim 33, wherein said delivery vehicle further carries a nucleic acid molecule encoding an immunogen.

38. The therapeutic composition of Claim 33, wherein said delivery vehicle further carries a peptide derived from *Mycobacterium tuberculosis*.

39. A therapeutic composition comprising a delivery vehicle carrying an isolated nucleic acid molecule encoding a superantigen, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences.

40. The therapeutic composition of Claim 39, wherein said delivery vehicle comprises a liposome.

41. The therapeutic composition of Claim 39, wherein said nucleic acid molecule encodes a superantigen selected

from the group consisting of SEA, SEB, SEC_1, SEC_2, SEC_3, SED, SEE and TSST.

42. The therapeutic composition of Claim 39, wherein said delivery vehicle further carries a nucleic acid molecule encoding a protein selected from the group consisting of a cytokine, a chemokine and an immunogen.

43. The therapeutic composition of Claim 39, wherein said delivery vehicle further carries a nucleic acid molecule encoding a protein selected from the group consisting of macrophage colony stimulating factor, macrophage colony stimulating factor, tumor necrosis factor α, interleukin-1, interleukin-2, interleukin-4, interleukin-6, interleukin-12, interleukin-15, C5a, IL-8, MIP1α, MIP1β, MCP-1, MCP-3, PAFR, FMLPR, LTB_4R, GRP, RANTES, eotaxin, lymphotactin, IP10, I-309, ENA78, GCP-2, NAP-2 and MGSA/gro.

44. The therapeutic composition of Claim 39, wherein said delivery vehicle further carries a peptide derived from *Mycobacterium tuberculosis*.

45. An adjuvant composition, comprising an immunogen and an isolated nucleic acid molecule encoding a superantigen, wherein said isolated nucleic acid molecule is operatively linked to one or more transcription control sequences.

46. The adjuvant composition of Claim 45, wherein said adjuvant composition further comprises a nucleic acid

molecule encoding a protein selected from the group consisting of a cytokine, a chemokine and mixtures thereof.

47. The adjuvant composition of Claim 45, wherein said immunogen comprises a compound selected from the group consisting of a nucleic acid molecule and a peptide.

48. The adjuvant composition of Claim 45, wherein said adjuvant composition comprises up to about 50% of a nucleic acid molecule encoding an immunogen and up to about 50% of a nucleic acid molecule encoding a superantigen.

49. The adjuvant composition of Claim 45, wherein said adjuvant composition comprises up to about 66% of a nucleic acid molecule encoding an immunogen and up to about 33% of a nucleic acid molecule encoding a superantigen.

50. The adjuvant composition of Claim 45, wherein said adjuvant composition comprises up to about 50% of a nucleic acid molecule encoding an immunogen, up to about 25% of a nucleic acid molecule encoding a superantigen and up to about 25% of a nucleic acid molecule selected from the group consisting of a nucleic acid molecule encoding a cytokine, a nucleic acid molecule encoding a chemokine and mixtures thereof.

51. The adjuvant composition of Claim 45, wherein said nucleic acid molecules comprise naked DNA.

52. The adjuvant composition of Claim 45, wherein said composition further comprises a peptide derived from *Mycobacterium tuberculosis*.

53. A method for increasing effector cell immunity in an animal, said method comprising administering to an animal an effective amount of an adjuvant composition comprising an immunogen and an isolated nucleic acid molecule encoding a superantigen.

54. The method of Claim 53, wherein said adjuvant composition further comprises a nucleic acid molecule encoding a protein selected from the group consisting of a cytokine, a chemokine and mixtures thereof.

55. The method of Claim 53, wherein said adjuvant composition further comprises a pharmaceutically acceptable carrier.

56. The method of Claim 53, wherein said step of administering comprises injecting said adjuvant composition by a route selected from the group consisting of intravenous, intraperitoneally, intramuscularly, intraarterially and directly into a specific tissue site.

57. The method of Claim 53, wherein said animal is a mammal.

58. The method of Claim 53, wherein said animal is selected from the from the group consisting of humans, horses, dogs, cats and cattle.

59. A method to treat an animal with cancer, said method comprising administering to an animal an effective amount of a therapeutic composition comprising: (a) an isolated nucleic acid molecule encoding a superantigen; and (b) an isolated nucleic acid molecule selected from the

group consisting of a nucleic acid molecule encoding a cytokine, a nucleic acid molecule encoding a chemokine, and mixtures thereof, wherein said nucleic acid molecules are operatively linked to one or more transcription control sequences.

60. The method of Claim 59, wherein said therapeutic composition further comprises a pharmaceutically acceptable carrier selected from the group consisting of an aqueous physiologically balanced solution, an artificial lipid-containing substrate, a natural lipid-containing substrate, an oil, an ester, a glycol, a virus and a metal particle.

61. The method of Claim 59, wherein said pharmaceutically acceptable carrier is selected from the group consisting of liposomes, micelles, cells and cellular membranes.

62. The method of Claim 59, wherein said pharmaceutically acceptable carrier comprises a liposome.

63. The method of Claim 59, wherein said pharmaceutically acceptable carrier comprises a liposome that includes a compound capable of specifically targeting said liposome to a tumor cell.

64. The method of Claim 63, wherein said compound is a tumor cell ligand.

65. The method of Claim 59, wherein said therapeutic composition is targeted to the site of a cancer in said animal by administering said therapeutic composition locally within the area of a cancer cell.

66. The method of Claim 59, wherein said cancer is selected from the group consisting of melanomas, squamous cell carcinoma, breast cancers, head and neck carcinomas, thyroid carcinomas, soft tissue sarcomas, bone sarcomas, testicular cancers, prostatic cancers, ovarian cancers, bladder cancers, skin cancers, brain cancers, angiosarcomas, hemangiosarcomas, mast cell tumors, primary hepatic cancers, lung cancers, pancreatic cancers, gastrointestinal cancers, renal cell carcinomas, hematopoietic neoplasias, leukemias and lymphomas.

67. A method to treat an animal with cancer, said method comprising introducing into a tumor cell *in vivo* an effective amount of a therapeutic composition comprising an isolated nucleic acid molecule encoding a superantigen, wherein said nucleic acid molecules are operatively linked to one or more transcription control sequences.

68. The method of Claim 67, wherein said isolated nucleic acid molecule encoding a superantigen comprises a recombinant molecule selected from the group consisting of PCR_3-SEB, PCR_3-SEA, PCR_3-SEB.S, PCR_3-SEA.S and PCR_3-TSST.

69. The method of Claim 67, wherein said therapeutic composition further comprises a pharmaceutically acceptable carrier comprising a liposome.

70. The method of Claim 67, wherein said therapeutic composition further comprises a pharmaceutically acceptable carrier comprising a liposome that includes a compound

capable of specifically targeting said liposome to a tumor cell.

71. The method of Claim 70, wherein said compound is a tumor cell ligand.

72. The method of Claim 67, wherein said therapeutic composition is targeted to the site of a cancer in said animal by administering said therapeutic composition locally within the area of a cancer cell.

73. The method of Claim 67, wherein said therapeutic composition is administered to said animal at a site comprising a lymph node.

74. A method to treat an animal with cancer, said method comprising introducing into a non-tumor cell an effective amount of a therapeutic composition comprising an isolated nucleic acid molecule encoding a superantigen, wherein said nucleic acid molecules are operatively linked to one or more transcription control sequences.

75. The method of Claim 74, wherein said step of administration is performed *in vivo*.

76. The method of Claim 74, wherein said isolated nucleic acid molecule encoding a superantigen comprises a recombinant molecule selected from the group consisting of PCR_3-SEB, PCR_3-SEA, PCR_3-SEB.S, PCR_3-SEA.S and PCR_3-TSST.

77. The method of Claim 74, wherein said therapeutic composition comprises a pharmaceutically acceptable carrier comprising a liposome.

78. The method of Claim 74, wherein said therapeutic composition is targeted to the site of a cancer in said animal by administering said therapeutic composition locally within the area of a cancer cell.

79. The method of Claim 74, wherein said therapeutic composition is administered to said animal at a site comprising a lymph node.

80. A method to treat an animal with cancer, said method comprising administering to an animal an effective amount of a therapeutic composition comprising: (a) an isolated nucleic acid molecule encoding a superantigen; and (b) an isolated nucleic acid molecule encoding a cytokine, wherein said nucleic acid molecules are operatively linked to one or more transcription control sequences, and wherein said therapeutic composition is targeted to the site of a cancer in said animal to treat said cancer.

81. The method of Claim 80, wherein said superantigen is selected from the group consisting of staphylococcal enterotoxins, retroviral antigens, streptococcal antigens, mycoplasma antigens, mycobacteria antigens, viral antigens and protozoan antigens.

82. The method of Claim 80, wherein said superantigen comprises staphylococcal enterotoxins.

83. The method of Claim 80, wherein said superantigen is selected from the group consisting of SEA, SEB, SEC_1, SEC_2, SEC_3, SED, SEE and TSST.

84. The method of Claim 80, wherein said superantigen is derived from a virus selected from the group consisting of mouse mammary tumor virus, rabies virus and herpes virus.

85. The method of Claim 80, wherein said cytokine is selected from the group consisting of hematopoietic growth factors, interleukins, interferons, immunoglobulin superfamily molecules, tumor necrosis factor family molecules and chemokines.

86. The method of Claim 80, wherein said cytokine is selected from the group consisting of granulocyte macrophage colony stimulating factor, macrophage colony stimulating factor, tumor necrosis factor α, interleukin-1, interleukin-6 and interleukin-12.

87. The method of Claim 80, wherein said cytokine is selected from the group consisting of granulocyte macrophage colony stimulating factor and tumor necrosis factor α.

88. The method of Claim 80, wherein said cytokine comprises granulocyte macrophage colony stimulating factor.

89. The method of Claim 80, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences capable of being expressed in a mammalian cell.

90. The method of Claim 80, wherein said transcription control sequences are selected from the group consisting of RSV control sequences, CMV control sequences,

retroviral LTR sequences, SV-40 control sequences and β-actin control sequences.

91. The method of Claim 80, wherein said isolated nucleic acid molecule comprises a recombinant molecule selected from the group consisting of PCR_3-SEB, PCR_3-SEA, PCR_3-SEB.S, PCR_3-SEA.S, PCR_3-TSST and PCR_3-GM_3.

92. The method of Claim 80, wherein said therapeutic composition further comprises a pharmaceutically acceptable carrier selected from the group consisting of an aqueous physiologically balanced solution, an artificial lipid-containing substrate, a natural lipid-containing substrate, an oil, an ester, a glycol, a virus and a metal particle.

93. The method of Claim 92, wherein said pharmaceutically acceptable carrier is selected from the group consisting of liposomes, micelles, cells and cellular membranes.

94. The method of Claim 92, wherein said pharmaceutically acceptable carrier comprises a liposome.

95. The method of Claim 92, wherein said pharmaceutically acceptable carrier comprises a liposome that includes a compound capable of specifically targeting said liposome to a tumor cell.

96. The method of Claim 95, wherein said compound is a tumor cell ligand.

97. The method of Claim 80, wherein said therapeutic composition is targeted to the site of a cancer in said

animal by administering said therapeutic composition locally within the area of a cancer cell.

98. The method of Claim 80, wherein said cancer is selected from the group consisting of melanomas, squamous cell carcinoma, breast cancers, head and neck carcinomas, thyroid carcinomas, soft tissue sarcomas, bone sarcomas, testicular cancers, prostatic cancers, ovarian cancers, bladder cancers, skin cancers, brain cancers, angiosarcomas, hemangiosarcomas, mast cell tumors, primary hepatic cancers, lung cancers, pancreatic cancers, gastrointestinal cancers, renal cell carcinomas, hematopoietic neoplasias, leukemias and lymphomas.

99. The method of Claim 80, wherein said cancer is selected from the group consisting of melanomas, lung cancers, thyroid carcinomas, breast cancers, renal cell carcinomas, squamous cell carcinomas, brain tumors and skin cancers.

100. The method of Claim 80, wherein said animal is selected from the group consisting of mammals and birds.

101. The method of Claim 80, wherein said animal is selected from the from the group consisting of humans, house pets, economic produce animals and zoo animals.

102. The method of Claim 80, wherein said animal is selected from the from the group consisting of humans, dogs, cats, sheep, cattle, horses and pigs.

103. A therapeutic composition comprising an isolated nucleic acid molecule encoding a superantigen and an

isolated nucleic acid molecule encoding a cytokine, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences.

104. The composition of Claim 103, wherein said therapeutic composition further comprises a pharmaceutically acceptable carrier.

105. The composition of Claim 104, wherein said pharmaceutically acceptable carrier is selected from the group consisting of an aqueous physiologically balanced solution, an artificial lipid-containing substrate, a natural lipid-containing substrate, an oil, an ester, a glycol, a virus and metal particles.

106. The composition of Claim 104, wherein said pharmaceutically acceptable carrier comprises a delivery vehicle capable of delivering said nucleic acid molecules to a targeted site in an animal.

107. The composition of Claim 106, wherein said delivery vehicle is selected from the group consisting of liposomes, micelles, cells and cellular membranes.

108. The composition of Claim 106, wherein said delivery vehicle comprises a liposome.

109. The composition of Claim 106, wherein said delivery vehicle comprises a tumor cell ligand.

110. The composition of Claim 103, wherein said superantigen is selected from the group consisting of staphylococcal enterotoxins, retroviral antigens,

streptococcal antigens, mycoplasma antigens, mycobacteria antigens, virus antigens and protozoan antigens.

111. The composition of Claim 103, wherein said superantigen comprises staphylococcal enterotoxins.

112. The composition of Claim 103, wherein said superantigen is selected from the group consisting of SEA, SEB, SEC_1, SEC_2, SEC_3, SED, SEE and TSST.

113. The composition of Claim 103, wherein said superantigen is derived from a virus selected from the group consisting of mouse mammary tumor virus, rabies virus and herpes virus.

114. The composition of Claim 103, wherein said cytokine is selected from the group consisting of hematopoietic growth factors, interleukins, interferons, immunoglobulin superfamily molecules, tumor necrosis factor family molecules and chemokines.

115. The composition of Claim 103, wherein said cytokine is selected from the group consisting of granulocyte macrophage colony stimulating factor, macrophage colony stimulating factor, tumor necrosis factor α, interleukin-1, interleukin-6 and interleukin-12.

116. The composition of Claim 103, wherein said cytokine is granulocyte macrophage colony stimulating factor and tumor necrosis factor α.

117. The composition of Claim 103, wherein said isolated nucleic acid molecules are operatively linked to

one or more transcription control sequences capable of being expressed in a mammalian cell.

118. The composition of Claim 103, wherein said transcription control sequences are selected from the group consisting of RSV control sequences, CMV control sequences, retroviral LTR control sequences, SV-40 control sequences and β-actin control sequences.

119. The composition of Claim 103, wherein said nucleic acid molecule comprises a recombinant molecule selected from the group consisting of PCR_3-SEB, PCR_3-SEA, PCR_3-SEB.S, PCR_3-SEA.S, PCR_3-TSST and PCR_3-GM_3.

120. The composition of Claim 103, wherein said therapeutic composition is useful for treating a cancer selected from the group consisting of melanomas, squamous cell carcinoma, breast cancers, head and neck carcinomas, thyroid carcinomas, soft tissue sarcomas, bone sarcomas, testicular cancers, prostatic cancers, ovarian cancers, bladder cancers, skin cancers, brain cancers, angiosarcomas, hemangiosarcomas, mast cell tumors, primary hepatic cancers, lung cancers, pancreatic cancers, gastrointestinal cancers, renal cell carcinomas, hematopoietic neoplasias, leukemias and lymphomas.

121. The composition of Claim 103, wherein said therapeutic composition is useful for treating a cancer selected from the group consisting of melanomas, lung cancers, thyroid carcinomas, breast cancers, renal cell

carcinomas, squamous cell carcinomas, brain tumors and skin cancers.

122. A recombinant molecule comprising an isolated nucleic acid molecule encoding a superantigen and an isolated nucleic acid molecule encoding a cytokine, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences.

123. The molecule of Claim 122, wherein said recombinant molecule is capable being expressed in a mammalian cell.

124. The molecule of Claim 122, wherein said recombinant molecule contains a transcription control sequence selected from the group consisting of RSV control sequences, CMV control sequences, retroviral LTR control sequences, SV-40 control sequences and β-actin control sequences.

125. The molecule of Claim 122, wherein said superantigen-encoding nucleic acid molecule encodes a toxin selected from the group consisting of staphylococcal enterotoxins, retroviral antigens, streptococcal antigens, mycoplasma antigens, mycobacteria antigens, virus antigens and protozoan antigens.

126. The molecule of Claim 122, wherein said superantigen-encoding nucleic acid molecule encodes a toxin selected from the group consisting of a SEA, SEB, SEC_1, SEC_2, SEC_3, SED, SEE and TSST gene.

127. The molecule of Claim 122, wherein said superantigen-encoding nucleic acid molecule lacks a bacterial leader sequence.

128. The molecule of Claim 122, wherein said recombinant molecule is selected from the group consisting of PCR_3-SEB, PCR_3-SEA, PCR_3-SEB.S, PCR_3-SEA.S, PCR_3-TSST and PCR_3-GM_3.

129. A therapeutic composition comprising a delivery vehicle carrying an isolated nucleic acid molecule encoding a superantigen and an isolated nucleic acid molecule encoding a cytokine, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences.

130. The composition of Claim 129, wherein said delivery vehicle is selected from the group consisting of a liposome, a micelle, a cell and a cellular membrane.

131. The composition of Claim 129, wherein said delivery vehicle comprises a liposome.

132. The composition of Claim 129, wherein said delivery vehicle comprises DOTMA and DOPE.

133. The composition of Claim 132, wherein said compound is a tumor cell ligand.

134. A method for increasing effector cell immunity in an animal, said method comprising administering to an animal an effective amount of a therapeutic composition comprising:

a) an isolated nucleic acid molecule encoding a superantigen; and

b) an isolated nucleic acid molecule encoding a cytokine,

wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences, and wherein said therapeutic composition is targeted to a site in said animal that contains an abnormal cell.

135. The method of Claim 134, wherein said abnormal cell is selected from the group consisting of a cancer cell, a cell infected with an infectious agent and a non-cancerous cell having abnormal proliferative growth.

136. The method of Claim 134, wherein said abnormal cell is a cancer cell.

137. The method of Claim 134, wherein said site is a tumor.

138. A method to suppress T cell activity in an animal, said method comprising administering to an animal an effective amount of a therapeutic composition comprising:

a) a naked isolated nucleic acid molecule encoding a superantigen; and

b) a pharmaceutically acceptable carrier,

wherein said isolated nucleic acid molecule is operatively linked to a traynscription control sequence, and wherein said therapeutic composition is targeted to a

site in said animal that contains excessive T cell activity.

139. The method of Claim 138, wherein said composition is capable of suppressing said T cell activity for at least about 6 weeks in said animal.

140. The method of Claim 138, wherein said composition is capable of suppressing said T cell activity for at least about 8 weeks in said animal.

141. The method of Claim 138, wherein said composition is capable of suppressing said T cell activity for about 10 weeks in said animal.

142. The method of Claim 138, wherein said carrier comprises an aqueous physiologically balanced solution.

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A. CLASSIFICATION OF SUBJECT MATTER

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B. FIELDS SEARCHED

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, EMBASE, BIOSIS, CAPLUS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 95/00178 A1 (BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM) 05 January 1995, see entire document.	1-142
Y	DONNELLY et al. Protective Efficacy of Intramuscular Immunization with Naked DNA. Ann. N.Y. Acad. Sci. 1995, Vol.772, pages 40-46, see entire document.	1-142
Y	BLACKMAN et al. In Vivo Effects of Superantigens. Life Sciences. 1995, Vol.57, No.19, pages 1717-1735, see entire document.	1-142
Y	MIETHKE et al. Superantigen Mediated Shock: A Cytokine Release Syndrome. Immunobiol. 1993, Vol.189, pages 270-284, see entire document.	1-142

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages.	Relevant to claim No.
Y	MIETHKE et al. Superantigens: The Paradox of T-Cell Activation versus Inactivation. Int. Arch. Allergy Immunol. 1995, Vol.106, pages 3-7, see entire document.	1-142
Y	LIU et al. Overview of DNA Vaccines. Ann. N.Y. Acad. Sci. 1995, Vol.772, pages 15-20, see entire document.	1-142

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(54) Title: GENE THERAPY FOR EFFECTOR CELL REGULATION

(57) Abstract

The present invention provides a nucleic acid-based therapeutic composition to treat an animal with disease by controlling the activity of effector cells, including T cells, macrophages, monocytes and/or natural killer cells, in the animal. Therapeutic compositions of the present invention include superantigen-encoding nucleic acid molecules, either in the presence or absence of a cytokine-encoding nucleic acid molecule and/or chemokine-encoding nucleic acid molecules, depending upon the disease being treated. The present invention also relates to an adjuvant for use with nucleic acid-based vaccines. Adjuvant compositions of the present invention include an immunogen combined with superantigen-encoding nucleic acid molecules, either in the presence or absence of a cytokine-encoding nucleic acid molecule and/or chemokine-encoding nucleic acid molecules.

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GENE THERAPY FOR EFFECTOR CELL REGULATION

FIELD OF THE INVENTION

The present invention relates to a product and process for regulating T cell activity by providing a superantigen gene, in the presence or absence of a cytokine and/or chemokine gene. The present invention also relates to a product and process for regulating T cell activity by providing a peptide and a superantigen gene, in the presence or absence of a cytokine and/or chemokine gene. In particular, the present invention relates to a product and process for controlling tumor development, immune responses to infectious diseases and diseases caused by immunological disorders.

BACKGROUND OF THE INVENTION

Two major causes of disease include infectious agents and malfunctions of normal biological functions of an animal. Examples of infectious agents include viruses, bacteria, parasites, yeast and other fungi. Examples of abnormal biological function include uncontrolled cell growth, abnormal immune responses and abnormal inflammatory responses. Traditional reagents used attempt to protect an animal from disease include reagents that destroy infectious agents or cells involved in deregulated biological functions. Such reagents, however, can result in unwanted side effects. For example, anti-viral drugs that disrupt the replication of viral DNA also often disrupt DNA replication in normal cells in the treated patient. Other treatments with chemotherapeutic reagents to

destroy cancer cells typically leads to side effects, such as bleeding, vomiting, diarrhea, ulcers, hair loss and increased susceptibility to secondary cancers and infections.

An alternative method of disease treatment includes modulating the immune system of a patient to assist the patient's natural defense mechanisms. Traditional reagents and methods used to attempt to regulate an immune response in a patient also result in unwanted side effects and have limited effectiveness. For example, immunosuppressive reagents (e.g., cyclosporin A, azathioprine, and prednisone) used to treat patients with autoimmune disease also suppress the patient's entire immune response, thereby increasing the risk of infection. In addition, immunopharmacological reagents used to treat cancer (e.g., interleukins) are short-lived in the circulation of a patient and are ineffective except in large doses. Due to the medical importance of immune regulation and the inadequacies of existing immunopharmacological reagents, reagents and methods to regulate specific parts of the immune system have been the subject of study for many years.

Stimulation or suppression of the immune response in a patient can be an effective treatment for a wide variety of medical disorders. T lymphocytes (T cells) are one of a variety of distinct cell types involved in an immune response. The activity of T cells is regulated by antigen, presented to a T cell in the context of a major

histocompatibility complex (MHC) molecule. The T cell receptor (TCR) then binds to the MHC:antigen complex. Once antigen is complexed to MHC, the MHC:antigen complex is bound by a specific TCR on a T cell, thereby altering the activity of that T cell.

The use of certain staphylococcal enterotoxin proteins that are capable of complexing with MHC molecules to influence T cell function has been suggested by various investigators, including, for example, White et al., *Cell* 56:27-35, 1989; Rellahan et al. *J. Expt. Med.* 172:1091-1100, 1990; Micusan et al., *Immunology* 5:3-11, 1993; Hermann et al., *Immunology* 5:33-39, 1993; Bhardwaj et al., *J. Expt. Med.* 178:633-642, 1993; and Kalland et al., *Med. Oncol. & Tumor Pharmacother.*, 10:37-47, 1993. In particular, various investigators have suggested that *Staphylococcal enterotoxin* proteins are useful for treating tumors, including Newell et al., *Proc. Natl. Acad. Sci. USA* 88:1074-1078, 1991; Kalland et al., PCT Application No. WO 91/04053, published April 4, 1991; Dohlstein et al., *Proc. Natl. Acad. Sci. USA* 88:9287-9291, 1991; Hedlund et al., *Cancer Immunol. Immunother.* 36:89-93, 1993; Lando et al., *Cancer Immunol. Immunother.* 36:223-228, 1993; Lukacs et al., *J. Exp. Med.* 178:343-348, 1993; Ochi et al., *J. Immunol.* 151:3180-3186, 1993; and Terman et al., PCT Application No. WO 93/24136, published December 9, 1993. These investigators, however, have only disclosed the use of bacterial enterotoxin proteins themselves. The use of

bacterial enterotoxin protein has the major disadvantage of being toxic to the recipient of the protein.

Thus, there is a need for a product and process that allows for the treatment of disease using bacterial enterotoxins in a non-toxic manner.

SUMMARY

Traditional pharmaceutical reagents used to treat cancer, infectious diseases and diseases caused by immunological disorders often have harmful side effects. In addition, such reagents can be unpredictable (e.g., treatment of cancer, vaccination against infectious agents). For example, chemotherapy and radiotherapy often cause extensive normal tissue damage during the process of treating cancerous tissue. In addition, vaccine treatments for the prevention or cure of infectious diseases are often ineffective because adjuvants useful in vaccine therapy are toxic to an animal.

The present invention is particularly advantageous in that it provides an effective therapeutic composition that enables the safe treatment of an animal with a reagent that is a potentially toxic an immunogenic protein. Upon delivery, expression of acid molecules contained in the therapeutic composition result in localized production of an effective but non-toxic amount of encoded proteins that may be toxic at concentrations that would be required if the encoded proteins were administered directly. The therapeutic compositions of the present invention can

provide long term expression of the encoded proteins at a site in an animal. Such long term expression allows for the maintenance of an effective, but non-toxic, dose of the encoded protein to treat a disease and limits the frequency of administration of the therapeutic composition needed to treat an animal. In addition, because of the lack of toxicity, therapeutic compositions of the present invention can be used in repeated treatments.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 illustrates the expression of superantigen-encoding DNA plasmids in mammalian cells.

Fig. 2 illustrates the proliferative response of canine PBMC's to canine melanoma cells transfected with a superantigen-encoding DNA plasmids.

Figs. 3A and 3B illustrate the release of superantigen protein by CHO cells transfected with superantigen-encoding DNA plasmids.

Fig. 4 illustrates the proliferative response of the Vβ3+ T cell clone AD10 to melanoma cells transfected with superantigen-encoding DNA plasmid.

Fig. 5 illustrates the release of canine GM-CSF by CHO cells transfected with GM-CSF-encoding DNA plasmid.

Figs. 6A and 6B illustrate the vaccination of mice with autologous tumor cells transfected with superantigen-encoding DNA plasmid.

Fig. 7 illustrates the effect of tumor target transfection on cytotoxic T cell lysis.

Fig. 8 illustrates the response of Vβ3+ T cells to intramuscular injection of a superantigen-encoding DNA plasmid.

Fig. 9 illustrates the antibody response resulting from the co-administration of DNA encoding an adjuvant and DNA encoding ovalbumin.

Fig. 10 illustrates that the co-administration of DNA encoding an adjuvant and DNA encoding ovalbumin increase interferon-gamma release from T cells restimulated *in vitro* by the ovalbumin protein.

Fig. 11 illustrates that the co-administration of DNA encoding an adjuvant and DNA encoding ovalbumin increase T cell proliferative responses to ovalbumin.

Fig. 12 illustrates that the co-administration of DNA encoding an adjuvant and DNA encoding ovalbumin increases CTL responses to ovalbumin.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a novel product and process for controlling effector cell activity. The present invention also relates to a novel adjuvant useful for enhancing an immune response. It is now known for the first time that a composition containing nucleic acid molecules encoding a superantigen, rather than superantigen proteins, is an effective therapeutic reagent for treating disease and is an effective adjuvant for enhancing an immune response. As used herein, a disease refers to any biological abnormality that is not beneficial to a subject.

The present inventors have also discovered that administration of a combination of nucleic acid molecules encoding: (1) a superantigen; (2) a superantigen and a cytokine; or (3) a superantigen and a chemokine, can act synergistically to effectively treat cancer and infectious disease. The present invention includes therapeutic compositions comprising: (a) an isolated nucleic acid molecule encoding a superantigen; or (b) an isolated nucleic acid molecule encoding a superantigen in combination with an isolated nucleic acid molecule encoding a cytokine and/or an isolated nucleic acid molecule encoding a chemokine. Administration of a therapeutic composition of the present invention to an animal results in the production of superantigen, cytokine or chemokine proteins, referred to herein as "encoded proteins." Each of the components of a therapeutic composition of the present invention is described in detail below, followed by a description of the methods by which the therapeutic composition is used and delivered.

One embodiment of the present invention includes a method for increasing effector cell immunity in an animal, the method comprising administering to an animal an effective amount of a therapeutic composition comprising: (a) an isolated nucleic acid molecule encoding a superantigen; or (b) an isolated nucleic acid molecule encoding a superantigen in combination with an isolated nucleic acid molecule encoding a cytokine and/or an isolated nucleic acid molecule encoding chemokine.

According to the present embodiment, the nucleic acid molecules are operatively linked to one or more transcription control sequences and the therapeutic composition is targeted to a site in the animal that contains an abnormal cell. According to the present invention, an effector cell, includes a helper T cell, a cytotoxic T cell, a macrophage, a monocyte and/or a natural killer cell. For example, the method of the present invention can be performed to increase the number of effector cells in an animal that are capable of killing or releasing cytokines or chemokines when presented with antigens derived from an abnormal cell or a pathogen. An effective amount of a therapeutic composition of the present invention comprises an amount capable of treating a disease as described herein. Alternatively, a method of the present invention can be performed to decrease the number of T cells found in a T cell subset that is preferentially stimulated and expanded by an autoantigen.

As used herein, effector cell immunity refers to increasing the number and/or the activity of effector cells in the area of the abnormal cell. In particular, T cell activity refers to increasing the number and/or the activity of T cells in the area of the abnormal cell. Also, as used herein, an abnormal cell refers to a cell displaying abnormal biological function, such as abnormal growth, development or death. Abnormal cells of the present invention, preferably includes cancer cells, cells infected with an infectious agent (i.e., a pathogen) and non-

cancerous cells having abnormal proliferative growth (e.g., sarcoidosis, granulomatous disease or papillomas) and with cancer cells and infected cells. Another embodiment of the present invention is a method to treat an animal with cancer, the method comprising administering to an animal an effective amount of a therapeutic composition comprising: (a) a nucleic acid molecule encoding a superantigen; or (b) a nucleic acid molecule encoding a superantigen in combination with an isolated nucleic acid molecule encoding a cytokine and/or a nucleic acid molecule encoding a chemokine. According to the present embodiment, the nucleic acid molecules are operatively linked to one or more transcription control sequences and the therapeutic composition is targeted to the site of a cancer.

One embodiment of a therapeutic composition of the present invention comprises an isolated nucleic acid molecule encoding a superantigen (also referred to herein as a "superantigen-encoding" nucleic acid molecule). Another embodiment of a therapeutic composition of the present invention comprises an isolated nucleic acid molecule encoding a superantigen, combined with an isolated nucleic acid molecule encoding a cytokine (also referred to herein as a "cytokine-encoding" nucleic acid molecule) and/or a nucleic acid molecule encoding a chemokine (also referred to as a "chemokine-encoding" nucleic acid molecule). According to these embodiments, the nucleic acid molecules are operatively linked to one or more transcription control sequences. It is to be noted that

the term "a" or "an" entity refers to one or more of that entity; for example, a compound refers to one or more compounds. As such, the terms "a" (or "an"), "one or more" and "at least one" can be used interchangeably herein. According to the present invention, an isolated, or biologically pure, nucleic acid molecule, is a nucleic acid molecule that has been removed from its natural milieu. As such, "isolated" and "biologically pure" do not necessarily reflect the extent to which the nucleic acid molecule has been purified. An isolated nucleic acid molecule can include DNA, RNA, or derivatives of either DNA or RNA. An isolated superantigen or cytokine nucleic acid molecule can be obtained from its natural source, either as an entire (i.e., complete) gene or a portion thereof capable of encoding a superantigen protein capable of binding to an MHC molecule or a cytokine protein capable of binding to a complementary cytokine receptor. A nucleic acid molecule can also be produced using recombinant DNA technology (e.g., polymerase chain reaction (PCR) amplification, cloning) or chemical synthesis. Nucleic acid molecules include natural nucleic acid molecules and homologues thereof, including, but not limited to, natural allelic variants and modified nucleic acid molecules in which nucleotides have been inserted, deleted, substituted, and/or inverted in such a manner that such modifications do not substantially interfere with the nucleic acid molecule's ability to encode a functional superantigen or a functional cytokine of the present invention.

A nucleic acid molecule homologue can be produced using a number of methods known to those skilled in the art (see, for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press, 1989). For example, nucleic acid molecules can be modified using a variety of techniques including, but not limited to, classic mutagenesis techniques and recombinant DNA techniques, such as site-directed mutagenesis, chemical treatment of a nucleic acid molecule to induce mutations, restriction enzyme cleavage of a nucleic acid fragment, ligation of nucleic acid fragments, polymerase chain reaction (PCR) amplification and/or mutagenesis of selected regions of a nucleic acid sequence, synthesis of oligonucleotide mixtures and ligation of mixture groups to "build" a mixture of nucleic acid molecules and combinations thereof. Nucleic acid molecule homologues can be selected from a mixture of modified nucleic acids by screening for the function of the protein encoded by the nucleic acid (e.g., superantigen, cytokine or chemokine activity, as appropriate). Techniques to screen for superantigen, cytokine or chemokine activity are known to those of skill in the art.

Although the phrase "nucleic acid molecule" primarily refers to the physical nucleic acid molecule and the phrase "nucleic acid sequence" primarily refers to the sequence of nucleotides on the nucleic acid molecule, the two phrases can be used interchangeably, especially with respect to a nucleic acid molecule, or a nucleic acid sequence, being

capable of encoding a superantigen, a cytokine or a chemokine protein. In addition, the phrase "recombinant molecule" primarily refers to a nucleic acid molecule operatively linked to a transcription control sequence, but can be used interchangeably with the phrase "nucleic acid molecule" which is administered to an animal. As heretofore disclosed, superantigen or cytokine proteins of the present invention include, but are not limited to, proteins having full-length superantigen, cytokine or chemokine coding regions, proteins having partial superantigen regions capable of binding to an MHC molecule, cytokine coding regions capable of binding to a complementary cytokine receptor, chemokine coding regions capable of binding to a complementary chemokine receptor, fusion proteins and chimeric proteins comprising combinations of different superantigens, cytokines and/or chemokines.

One embodiment of the present invention is an isolated superantigen-encoding nucleic acid molecule that encodes at least a portion of a full-length superantigen, or a homologue of a superantigen. As used herein, "at least a portion of a superantigen" refers to a portion of a superantigen protein capable of binding to an MHC molecule in such a manner that a TCR can bind to the resulting superantigen:MHC complex. Preferably, a superantigen nucleic acid molecule of the present invention encodes an entire coding region of a superantigen, and more preferably the coding region absent a leader sequence. Production of a truncated superantigen protein lacking a bacterial leader

sequence is preferred to enhance secretion of the superantigen from a cell. As used herein, a homologue of a superantigen is a protein having an amino acid sequence that is sufficiently similar to a natural superantigen amino acid sequence that a nucleic acid sequence encoding the homologue encodes a protein capable of binding to an MHC molecule.

In accordance with the present invention, a superantigen comprises a family of T cell regulatory proteins that are capable of binding both to an MHC molecule. A superantigen binds to the extracellular portion of an MHC molecule to form and MHC:superantigen complex. The activity of a T cell can be modified when a TCR binds to an MHC:superantigen complex. Under certain circumstances, an MHC:superantigen complex can have a mitogenic role (i.e., the ability to stimulate the proliferation of T cells) or a suppressive role (i.e., deletion of T cell subsets). The ability of an MHC:superantigen complex to have a stimulatory or suppressive role can depend upon factors, such as the concentration and environment (i.e., tissue location and/or the presence of cytokines).

The mitogenic role of a superantigen is distinct from other known mitogens (e.g., lectins derived from plants) in that superantigens are capable of stimulating the proliferation of particular subsets of T cells having TCR's that specifically bind to the superantigen. For example, a superantigen, when added to a mixed lymphocyte

population, is able to stimulate the proliferation of a select population of T cells from the mixed population of cells. Examples of T cell subsets stimulated by superantigens complexed with MHC molecules include T cells expressing a TCR comprising mouse $V_\beta 1$, $V_\beta 3$, $V_\beta 7$, $V_\beta 8.1$, $V_\beta 8.2$, $V_\beta 8.3$, $V_\beta 10$, $V_\beta 11$, $V_\beta 17$, $V_\beta 15$ or $V_\beta 16$ chains, and T cells expressing a TCR comprising human $V_\beta 1.1$, $V_\beta 2$, $V_\beta 3$, $V_\beta 5$, $V_\beta 6$, $V_\beta 7.3$, $V_\beta 7.4$, $V_\beta 9.1$, $V_\beta 12$, $V_\beta 14$, $V_\beta 15$, $V_\beta 17$ or $V_\beta 20$ chains.

A superantigen-encoding nucleic acid molecule of the present invention preferably encodes superantigens that includes, but is not limited to, staphylococcal enterotoxins, retroviral antigens, streptococcal antigens, mycoplasma antigens, mycobacterium antigens, viral antigens (e.g., a superantigen from mouse mammary tumor virus, rabies virus or herpes virus) and endoparasitic antigens (e.g., protozoan or helminth antigens), more preferably staphylococcal enterotoxins, and even more preferably *Staphylococcal enterotoxin* A (SEA), *Staphylococcal enterotoxin* B (SEB), *Staphylococcal enterotoxin* C_1 (SEC_1), *Staphylococcal enterotoxin* C_2 (SEC_2), *Staphylococcal enterotoxin* C_3 (SEC_3), *Staphylococcal enterotoxin* D (SED), *Staphylococcal enterotoxin* E (SEE) and Toxic Shock Syndrome Toxin (TSST).

A preferred nucleic acid molecule encoding a *Staphylococcal enterotoxin* of the present invention comprises a nucleic acid sequence represented by SEQ ID NO:1 (representing a full-length SEB gene), SEQ ID NO:3

(representing a full-length SEA gene) or SEQ ID NO:6 (representing a full-length TSST gene). A preferred *Staphylococcal enterotoxin* protein of the present invention comprises an amino acid sequence represented by SEQ ID NO:2 (representing a full-length SEB protein), SEQ ID NO:4 (representing a full-length SEA protein) or SEQ ID NO:7 (representing a full-length TSST protein).

In a preferred embodiment, a nucleic acid molecule of the present invention encoding a superantigen comprises a nucleic acid sequence spanning base pair 46 to at least base pair 768 of SEQ ID NO:1, a nucleic acid sequence spanning base pair 46 to about base pair 751 of SEQ ID NO:3 or SEQ ID NO:6.

Another embodiment of the present invention includes a cytokine-encoding nucleic acid molecule that encodes a full-length cytokine or a homologue of the cytokine protein. As used herein, a homologue of a cytokine is a protein having an amino acid sequence that is sufficiently similar to a natural cytokine amino acid sequence so as to have cytokine activity. In accordance with the present invention, a cytokine includes a protein that is capable of affecting the biological function of another cell. A biological function affected by a cytokine can include, but is not limited to, cell growth, cell differentiation or cell death. Preferably, a cytokine of the present invention is capable of binding to a specific receptor on the surface of a cell, thereby affecting the biological function of a cell.

A cytokine-encoding nucleic acid molecule of the present invention encodes a cytokine that is capable of affecting the biological function of a cell, including, but not limited to, a lymphocyte, a muscle cell, a hematopoietic precursor cell, a mast cell, a natural killer cell, a macrophage, a monocyte, an epithelial cell, an endothelial cell, a dendritic cell, a mesenchymal cell, a Langerhans cell, cells found in granulomas and tumor cells of any cellular origin, and more preferably a mesenchymal cell, an epithelial cell, an endothelial cell, a muscle cell, a macrophage, a monocyte, a T cell and a dendritic cell.

A preferred cytokine nucleic acid molecule of the present invention encodes a hematopoietic growth factor, an interleukin, an interferon, an immunoglobulin superfamily molecule, a tumor necrosis factor family molecule and/or a chemokine (i.e., a protein that regulates the migration and activation of cells, particularly phagocytic cells). A more preferred cytokine nucleic acid molecule of the present invention encodes a granulocyte macrophage colony stimulating factor (GM-CSF), tumor necrosis factor α (TNF-α), macrophage colony stimulating factor (M-CSF), interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-12 (IL-12), interleukin-15 (IL-15) and/or IGIF. An even more preferred cytokine nucleic acid molecule of the present invention encodes GM-CSF, IL-2, IL-12, IGIF and/or TNF-α, with GM-CSF being even more preferred.

As will be apparent to one of skill in the art, the present invention is intended to apply to cytokines derived from all types of animals. A preferred animal from which to derive cytokines includes a mouse, a human, a cat and a dog. A more preferred animal from which to derive cytokines includes a cat, a dog and a human. An even more preferred animal from which to derive cytokines is a human.

According to the present invention, a cytokine-encoding nucleic acid molecule of the present invention is derived from the same species of animal as the animal to be treated. For example, a cytokine-encoding nucleic acid molecule derived from a canine (i.e., dog) nucleic acid molecule is used to treat a disease in a canine. Thus, a preferred cytokine-encoding nucleic acid molecule of the present invention comprises a nucleic acid molecule encoding human GM-CSF, as described in the art. A human GM-CSF-encoding nucleic acid molecule of the present invention can be produced using methods standard PCR amplification methods with primers designed from the human GM-CSF nucleic acid sequence disclosed in Nash (*Blood* 78:930, 1991). Such PCR products can be cloned into a PCR_3 expression vector using the methods generally described in Example 1.

Another embodiment of the present invention includes a chemokine-encoding nucleic acid molecule that encodes a full-length chemokine or a homologue of the chemokine protein. As used herein, a homologue of a chemokine is a protein having an amino acid sequence that is sufficiently

similar to a natural chemokine amino acid sequence so as to have chemokine activity. In accordance with the present invention, a chemokine includes a protein that is capable of attracting cells involved in an immune response (immunologic cells), including phagocytic cells. For example, immunologic cells are recruited from the blood to a site at which the chemokine is located (e.g., a site of infection). Preferably, a chemokine of the present invention is capable of binding to a specific receptor on the surface of a cell, thereby attracting the cell to a specific location.

A chemokine-encoding nucleic acid molecule of the present invention encodes a chemokine that is capable of attracting a cell to a site, including, but not limited to, a dendritic cell, a neutrophil, a macrophage, a T lymphocyte and Langerhans cells, and more preferably a dendritic cell, a macrophage and a T lymphocyte.

A preferred chemokine-encoding nucleic acid molecule of the present invention encodes an α-chemokine or a β-chemokine. A more preferred chemokine-encoding nucleic acid molecule of the present invention encodes a C5a, interleukin-8 (IL-8), monocyte chemotactic protein 1α (MIP1α), monocyte chemotactic protein 1β (MIP1β), monocyte chemoattractant protein 1 (MCP-1), monocyte chemoattractant protein 3 (MCP-3), platelet activating factor (PAFR), N-Formyl-methionyl-leucyl-[^{3H}]phenylalanine (FMLPR), leukotriene B_4 (LTB_4R), gastrin releasing peptide (GRP), RANTES, eotaxin, lymphotactin, IP10, I-309, ENA78, GCP-2,

NAP-2 and/or MGSA/gro. An even more preferred chemokine-encoding nucleic acid molecule of the present invention encodes IL-8, MIP1α, MIP1β, MCP-1, MCP-3, RANTES and/or NAP-2, with IL-8, Rantes, MIP1α and/or MIP1β being even more preferred.

As will be apparent to one of skill in the art, the present invention is intended to apply to chemokines derived from all types of animals. Preferred animals from which to derive chemokines includes mice, humans, dogs, cats, cattle and horses. More preferred animals from which to derive chemokines includes dogs, cats, humans and cattle. Even more preferred animals from which to derive chemokines are humans.

According to the present invention, a chemokine-encoding nucleic acid molecule of the present invention is derived from the same species of animal as the animal to be treated. For example, a chemokine-encoding nucleic acid molecule derived from a canine (i.e., dog) nucleic acid molecule is used to treat a disease in a canine. Thus, a preferred chemokine-encoding nucleic acid molecule of the present invention comprises a nucleic acid molecule encoding a dog, cat, human, bovine and/or equine chemokine. Preferred nucleic acid molecules of the present invention encode IL-8, Rantes, MIP1α and/or MIP1β, as described in the art. For example, a human MIP1α-encoding nucleic acid molecule of the present invention can be produced using standard PCR amplification methods with primers designed from the human MIP1α-encoding nucleic acid sequence

disclosed in the art. Such PCR products can be cloned into a PCR_3 expression vector using the methods generally described in Example 1.

The present invention includes a nucleic acid molecule of the present invention operatively linked to one or more transcription control sequences to form a recombinant molecule. The phrase "operatively linked" refers to linking a nucleic acid molecule to a transcription control sequence in a manner such that the molecule is able to be expressed when transfected (i.e., transformed, transduced or transfected) into a host cell. Transcription control sequences are sequences which control the initiation, elongation, and termination of transcription. Particularly important transcription control sequences are those which control transcription initiation, such as promoter, enhancer, operator and repressor sequences. Suitable transcription control sequences include any transcription control sequence that can function in at least one of the recombinant cells of the present invention. A variety of such transcription control sequences are known to those skilled in the art. Preferred transcription control sequences include those which function in animal, bacteria, helminth, insect cells, and preferably in animal cells. More preferred transcription control sequences include, but are not limited to, simian virus 40 (SV-40), β-actin, retroviral long terminal repeat (LTR), Rous sarcoma virus (RSV), cytomegalovirus (CMV), *tac*, *lac*, *trp*, *trc*, oxy-pro, omp/lpp, rrnB, bacteriophage lambda (λ) (such as λp_L and λp_R

and fusions that include such promoters), bacteriophage T7, T7*lac*, bacteriophage T3, bacteriophage SP6, bacteriophage SP01, metallothionein, alpha mating factor, *Pichia* alcohol oxidase, alphavirus subgenomic promoters (such as Sindbis virus subgenomic promoters), baculovirus, *Heliothis zea* insect virus, vaccinia virus and other poxviruses, herpesvirus, and adenovirus transcription control sequences, as well as other sequences capable of controlling gene expression in eukaryotic cells. Additional suitable transcription control sequences include tissue-specific promoters and enhancers (e.g., tumor cell-specific enhancers and promoters), and inducible promoters (e.g., tetracycline). Transcription control sequences of the present invention can also include naturally occurring transcription control sequences naturally associated with a gene encoding a superantigen, a cytokine or a chemokine of the present invention.

Recombinant molecules of the present invention, which can be either DNA or RNA, can also contain additional regulatory sequences, such as translation regulatory sequences, origins of replication, and other regulatory sequences that are compatible with the recombinant cell. In one embodiment, a recombinant molecule of the present invention also contains secretory signals (i.e., signal segment nucleic acid sequences) to enable an expressed superantigen, cytokine or a chemokine protein to be secreted from the cell that produces the protein. Suitable signal segments include: (1) a bacterial signal segment, in

particular a superantigen signal segment; (2) a cytokine signal segment; (3) a chemokine signal segment; (4) or any heterologous signal segment capable of directing the secretion of a superantigen, cytokine and/or chemokine protein of the present invention. Preferred signal segments include, but are not limited to, signal segments associated with SEB, SEA, TSST, GM-CSF, M-CSF, TNFα, IL-1, IL-2, IL-4, IL-6, IL-12, IL-15, C5a, IGIF, IL-8, MIP1α, MIP1β, MCP-1, MCP-3, PAFR, FMLPR, LTB_4R, GRP, RANTES, eotaxin, lymphotactin, IP10, I-309, ENA78, GCP-2, NAP-2 and/or MGSA/gro protein.

Preferred recombinant molecules of the present invention include a recombinant molecule containing a nucleic acid molecule encoding a superantigen, a recombinant molecule containing a nucleic acid molecule encoding a cytokine, a recombinant molecule containing a nucleic acid molecule encoding a chemokine, a recombinant molecule containing a nucleic acid molecule encoding a superantigen and a nucleic acid molecule encoding a cytokine to form a chimeric recombinant molecule, or a recombinant molecule containing a nucleic acid molecule encoding a superantigen and a nucleic acid molecule encoding a chemokine to form a chimeric recombinant molecule. The nucleic acid molecules contained in such recombinant chimeric molecules are operatively linked to one or more transcription control sequences, in which each nucleic acid molecule contained in a chimeric recombinant molecule can be expressed using the same or different

regulatory control sequences. Preferred recombinant molecules of the present invention comprise a nucleic acid sequence represented by SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, or combinations thereof. Particularly preferred recombinant molecules include PCR_3-SEB, PCR_3-SEA, PCR_3-SEB.S, PCR_3-SEA.S, PCR_3-TSST and PCR_3-GM_3, the production of which is disclosed herein. Other preferred nucleic acid sequences include Rantes nucleic acid sequence (SEQ ID. NO:13), MIP1α nucleic acid sequence (see Davatelis et al., *J. Exp. Med.* 167:1939-1944, 1988) and MIP1β nucleic acid sequence (see Sherry et al., *J. Exp. Med.* 168:2251-2259, 1988).

According to the present invention, a recombinant molecule can be dicistronic. A cistron refers to a unit of DNA that is capable of encoding an amino acid sequence having a naturally-occurring biological function. A dicistronic plasmid refers to a plasmid containing 2 cistrons. Preferably, a dicistronic recombinant molecule of the present invention comprises an internal ribosome entry site (IRES) element to which eukaryotic ribosomes can bind (see, for example, Jang et al., *J. Virol.* 62:2636-2643, 1988; Pelletier et al. *Nature* 334:320-325, 1988; Jackson, *Nature* 353:14-15, 1991; Macejek et al., *Nature* 353:90-94, 1991; Oh et al., *Genes & Develop.* 6:1643-1653, 1992; Molla et al., *Nature* 356:255-257, 1992; and Kozak, *Crit. Rev. Biochem. Molec. Biol.* 27(4,5):385-402, 1992).

In one embodiment, a dicistronic recombinant molecule of the present invention comprises a eukaryotic promoter, operatively linked to a superantigen-encoding nucleic acid

molecule of the present invention and a cytokine-encoding nucleic acid molecule of the present invention separated by an IRES nucleic acid sequence, or a superantigen-encoding nucleic acid molecule of the present invention and chemokine-encoding nucleic acid molecule of the present invention separated by an IRES nucleic acid sequence.

In another embodiment, a dicistronic recombinant molecule of the present invention comprises a eukaryotic promoter, operatively linked to a first superantigen-encoding nucleic acid molecule of the present invention and a second superantigen-encoding nucleic acid molecule of the present invention separated by an IRES nucleic acid sequence. Preferably, a first superantigen-encoding nucleic acid molecule encodes a different superantigen than a second superantigen-encoding nucleic acid molecule.

One or more recombinant molecules of the present invention can be used to produce an encoded product (i.e., a superantigen protein, a cytokine and a chemokine protein) of the present invention. In one embodiment, an encoded product of the present invention is produced by expressing a nucleic acid molecule of the present invention in a cell under conditions effective to produce the protein. A preferred method to produce an encoded protein is by transforming (i.e., introducing a recombinant molecule into a cell in such a manner that the recombinant molecule is expressed by the cell) a host cell with one or more recombinant molecules of the present invention to form a recombinant cell. Suitable host cells to transform include

any cell into which a recombinant molecule can be introduced. Host cells can be either untransformed cells or cells that are already transformed with at least one nucleic acid molecule. Host cells of the present invention can be any cell capable of producing a superantigen, a cytokine and/or a chemokine of the present invention, including bacterial, fungal, animal parasite, insect and animal cells. A preferred host cell includes a mammalian and a bird cell. A more preferred host cell includes mammalian lymphocytes, muscle cells, hematopoietic precursor cells, mast cells, natural killer cells, macrophages, monocytes, epithelial cells, endothelial cells, dendritic cells, mesenchymal cells, Langerhans cells, cells found in granulomas and tumor cells of any cellular origin. An even more preferred host cell of the present invention includes mammalian mesenchymal cells, epithelial cells, endothelial cells, macrophages, monocytes, muscle cells, T cells and dendritic cells.

According to the present invention, a recombinant molecule can be introduced into a host cell *in vivo* (i.e., in an animal) or *in vitro* (i.e., outside of an animal, such as in tissue culture). Introduction of a nucleic acid molecule into a host cell can be accomplished by any method by which a nucleic acid molecule can be inserted into the cell. Transformation techniques include, but are not limited to, transfection, electroporation, microinjection, lipofection, adsorption, and protoplast fusion. Preferred methods to introduce a recombinant molecule into host cells

in vivo include lipofection and adsorption (discussed in detail below).

A recombinant cell of the present invention comprises a cell into which a nucleic acid molecule that encodes a superantigen, a cytokine and/or a chemokine has been introduced. In one embodiment, a recombinant cell of the present invention is transformed with a nucleic acid molecule that includes at least a portion of PCR_3-SEB, PCR_3-SEA, PCR_3-SEB.S, PCR_3-SEA.S, PCR_3-TSST, or combinations thereof. Particularly preferred recombinant cells include cells transformed with PCR_3-SEB, PCR_3-SEA, PCR_3-SEB.S, PCR_3-SEA.S or PCR_3-TSST, with PCR_3-SEB.S, PCR_3-SEA.S or PCR_3-TSST being even more preferred.

In another embodiment, a recombinant cell of the present invention is transformed with a nucleic acid molecule that includes at least a portion of PCR_3-SEB, PCR_3-SEA, PCR_3-SEB.S, PCR_3-SEA.S, PCR_3-TSST or combinations thereof, and PCR_3-GM_3. Particularly preferred stimulatory recombinant cells include cells transformed with PCR_3-SEA and PCR_3-GM_3, PCR_3-SEA.S and PCR_3-GM_3, PCR_3-SEB and PCR_3-GM_3, PCR_3-SEB.S and PCR_3-GM_3, or PCR_3-TSST and PCR_3-GM_3. Even more preferred stimulatory recombinant cells include cells transformed with PCR_3-SEB.S and PCR_3-GM_3, or PCR_3-SEA.S and PCR_3-GM_3, and PCR_3-TSST and PCR_3-GM_3.

Recombinant DNA technologies can be used to improve expression of transformed nucleic acid molecules by manipulating, for example, the number of copies of the nucleic acid molecules within a host cell, the efficiency

with which those nucleic acid molecules are transcribed, the efficiency with which the resultant transcripts are translated, and the efficiency of post-translational modifications. Recombinant techniques useful for increasing the expression of nucleic acid molecules of the present invention include, but are not limited to, operatively linking nucleic acid molecules to high-copy number plasmids, integration of the nucleic acid molecules into one or more host cell chromosomes, addition of vector stability sequences to plasmids, substitutions or modifications of transcription control signals (e.g., promoters, operators, enhancers), substitutions or modifications of translational control signals (e.g., ribosome binding sites, Shine-Dalgarno sequences), modification of nucleic acid molecules of the present invention to correspond to the codon usage of the host cell, and deletion of sequences that destabilize transcripts. The activity of an expressed recombinant protein of the present invention may be improved by fragmenting, modifying, or derivatizing nucleic acid molecules encoding such a protein.

Further embodiments of compositions of the present invention can also include a compound capable of inhibiting the downregulation of T cell activity. In particular, such a compound can include an inhibitor of CTLA-4. An inhibitor of CTLA-4 includes any compound capable of inhibiting the activity of CTLA-4 and/or inhibiting the binding of CTLA-4 to its natural ligand (e.g., B7).

Preferably, an inhibitor of CTLA-4 includes, but is not limited to a ligand of CTLA-4 or an analog (i.e., antagonist) of CTLA-4. Preferred ligands of CTLA-4 include: an antibody that specifically binds to CTLA-4 in such a manner that CTLA-4 activity is inhibited; at least a portion of a B7 molecule, in particular a B7 fusion protein; or a synthetic oligonucleotide that binds CTLA-4 protein. A preferred analog of CTLA-4 includes a molecule capable of binding to B7 in such a manner that B7 signal transduction is not activated and CTLA-4 binding to the B7 molecule is inhibited. It is within the scope of the invention that a CTLA-4 inhibitor can comprise a nucleic acid molecule, a protein or a synthetic chemical molecule when combined in a composition of the present invention.

In another embodiment of the present invention, a therapeutic composition further comprises a pharmaceutically acceptable carrier. As used herein, a "carrier" refers to any substance suitable as a vehicle for delivering a nucleic acid molecule of the present invention to a suitable *in vivo* or *in vitro* site. As such, carriers can act as a pharmaceutically acceptable excipient of a therapeutic composition containing a nucleic acid molecule of the present invention. Preferred carriers are capable of maintaining a nucleic acid molecule of the present invention in a form that, upon arrival of the nucleic acid molecule to a cell, the nucleic acid molecule is capable of entering the cell and being expressed by the cell. Carriers of the present invention include: (1) excipients

or formularies that transport, but do not specifically target a nucleic acid molecule to a cell (referred to herein as non-targeting carriers); and (2) excipients or formularies that deliver a nucleic acid molecule to a specific site in an animal or a specific cell (i.e., targeting carriers). Examples of non-targeting carriers include, but are not limited to water, phosphate buffered saline, Ringer's solution, dextrose solution, serum-containing solutions, Hank's solution, other aqueous physiologically balanced solutions, oils, esters and glycols. Aqueous carriers can contain suitable auxiliary substances required to approximate the physiological conditions of the recipient, for example, by enhancing chemical stability and isotonicity.

Suitable auxiliary substances include, for example, sodium acetate, sodium chloride, sodium lactate, potassium chloride, calcium chloride, and other substances used to produce phosphate buffer, Tris buffer, and bicarbonate buffer. Auxiliary substances can also include preservatives, such as thimerosal, m- and o-cresol, formalin and benzol alcohol. Preferred auxiliary substances for aerosol delivery include surfactant substances non-toxic to an animal, for example, esters or partial esters of fatty acids containing from about six to about twenty-two carbon atoms. Examples of esters include, caproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, olesteric, and oleic acids. Other carriers can include metal particles (e.g., gold particles) for use

with, for example, a biolistic gun through the skin. Therapeutic compositions of the present invention can be sterilized by conventional methods and/or lyophilized.

Targeting carriers are herein referred to as "delivery vehicles." Delivery vehicles of the present invention are capable of delivering a therapeutic composition of the present invention to a target site in an animal. A "target site" refers to a site in an animal to which one desires to deliver a therapeutic composition. For example, a target site can be a malignant tumor cell, a non-malignant tumor cell, a lymph node or a lesion caused by an infectious agent, or an area around such cell, tumor or lesion, which is targeted by direct injection or delivery using liposomes or other delivery vehicles. Examples of delivery vehicles include, but are not limited to, artificial and natural lipid-containing delivery vehicles. Natural lipid-containing delivery vehicles include cells and cellular membranes. Artificial lipid-containing delivery vehicles include liposomes and micelles. A delivery vehicle of the present invention can be modified to target to a particular site in an animal, thereby targeting and making use of a nucleic acid molecule of the present invention at that site. Suitable modifications include manipulating the chemical formula of the lipid portion of the delivery vehicle and/or introducing into the vehicle a compound capable of specifically targeting a delivery vehicle to a preferred site, for example, a preferred cell type. Specifically targeting refers to causing a delivery vehicle

to bind to a particular cell by the interaction of the compound in the vehicle to a molecule on the surface of the cell. Suitable targeting compounds include ligands capable of selectively (i.e., specifically) binding another molecule at a particular site. Examples of such ligands include antibodies, antigens, receptors and receptor ligands. For example, an antibody specific for an antigen found on the surface of a cancer cell can be introduced to the outer surface of a liposome delivery vehicle so as to target the delivery vehicle to the cancer cell. Tumor cell ligands include ligands capable of binding to a molecule on the surface of a tumor cell. Manipulating the chemical formula of the lipid portion of the delivery vehicle can modulate the extracellular or intracellular targeting of the delivery vehicle. For example, a chemical can be added to the lipid formula of a liposome that alters the charge of the lipid bilayer of the liposome so that the liposome fuses with particular cells having particular charge characteristics.

A preferred delivery vehicle of the present invention is a liposome. A liposome is capable of remaining stable in an animal for a sufficient amount of time to deliver a nucleic acid molecule of the present invention to a preferred site in the animal. A liposome of the present invention is preferably stable in the animal into which it has been administered for at least about 30 minutes, more preferably for at least about 1 hour and even more preferably for at least about 24 hours.

A liposome of the present invention comprises a lipid composition that is capable of targeting a nucleic acid molecule of the present invention to a particular, or selected, site in an animal. Preferably, the lipid composition of the liposome is capable of targeting to any organ of an animal, more preferably to the lung, liver, spleen, heart brain, lymph nodes and skin of an animal, and even more preferably to the lung of an animal.

A liposome of the present invention comprises a lipid composition that is capable of fusing with the plasma membrane of the targeted cell to deliver a nucleic acid molecule into a cell. Preferably, the transfection efficiency of a liposome of the present invention is at least about 0.5 microgram (μg) of DNA per 16 nanomole (nmol) of liposome delivered to about 10^6 cells, more preferably at least about 1.0 μg of DNA per 16 nmol of liposome delivered to about 10^6 cells, and even more preferably at least about 2.0 μg of DNA per 16 nmol of liposome delivered to about 10^6 cells.

A preferred liposome of the present invention is between about 100 and about 500 nanometers (nm), more preferably between about 150 and about 450 nm and even more preferably between about 200 and about 400 nm in diameter.

Suitable liposomes for use with the present invention include any liposome. Preferred liposomes of the present invention include those liposomes standardly used in, for example, gene delivery methods known to those of skill in the art. More preferred liposomes comprise liposomes

having a polycationic lipid composition and/or liposomes having a cholesterol backbone conjugated to polyethylene glycol. Even more preferred liposomes include liposomes produced according to the method described in Example 2.

In one embodiment, a liposome of the present invention comprises a compound capable of targeting the liposome to a tumor cell. Such a liposome preferably includes a tumor cell ligand exposed on the outer surface of the liposome.

Complexing a liposome with a nucleic acid molecule of the present invention can be achieved using methods standard in the art (see, for example, methods described in Example 2). A suitable concentration of a nucleic acid molecule of the present invention to add to a liposome includes a concentration effective for delivering a sufficient amount of nucleic acid molecule to a cell such that the cell can produce sufficient superantigen and/or cytokine protein to regulate effector cell immunity in a desired manner. Preferably, nucleic acid molecules are combined with liposomes at a ratio of from about 0.1 μg to about 10 μg of nucleic acid molecule of the present invention per about 8 nmol liposomes, more preferably from about 0.5 μg to about 5 μg of nucleic acid molecule per about 8 nmol liposomes, and even more preferably about 1.0 μg of nucleic acid molecule per about 8 nmol liposomes.

Another preferred delivery vehicle comprises a recombinant virus particle vaccine. A recombinant virus particle vaccine of the present invention includes a therapeutic composition of the present invention, in which

the recombinant molecules contained in the composition are packaged in a viral coat that allows entrance of DNA into a cell so that the DNA is expressed in the cell. A number of recombinant virus particles can be used, including, but not limited to, those based on alphaviruses, poxviruses, adenoviruses, herpesviruses, arena virus and retroviruses.

Another preferred delivery vehicle comprises a recombinant cell vaccine. Preferred recombinant cell vaccines of the present invention include tumor vaccines, in which allogeneic (i.e., cells derived from a source other than a patient, but that are histiotype compatible with the patient) or autologous (i.e., cells isolated from a patient) tumor cells are transfected with recombinant molecules contained in a therapeutic composition, irradiated and administered to a patient by, for example, intradermal, intravenous or subcutaneous injection. Therapeutic compositions to be administered by tumor cell vaccine, include recombinant molecules of the present invention without carrier. Tumor cell vaccine treatment is useful for the treatment of both tumor and metastatic cancer. Use of a tumor vaccine of the present invention is particular useful for treating metastatic cancer, including preventing metastatic disease, as well as, curing existing metastatic disease. Methods for developing and administering include those standard in the art (see for example, Dranoff et al., *Proc. Natl. Acad. Sci. USA* 90:3539-3543, 1993, which is incorporated herein by reference in its entirety).

A therapeutic composition of the present invention is useful for the treatment of a variety of diseases, including, but not limited to, cancer, autoimmune disease, infectious diseases, and other diseases that can be alleviated by either stimulating or suppressing T cell activity. As used herein, the term "treatment" refers to protecting an animal from a disease or alleviating a disease in an animal. A therapeutic composition of the present invention is advantageous for the treatment of cancer in that the composition overcomes the mechanisms by which cancer cells avoid immune elimination (i.e., by which cancer cells avoid the immune response effected by the animal in response to the disease). Cancer cells can avoid immune elimination by, for example, being only slightly immunogenic, modulating cell surface antigens and inducing immune suppression. Suitable therapeutic compositions for use in the treatment of cancer comprises a superantigen-encoding recombinant molecule; or a combination of a superantigen-encoding recombinant molecule, with a cytokine-encoding recombinant molecule and/or a chemokine recombinant molecule of the present invention. Preferred therapeutic compositions for use in the treatment of cancer comprises a superantigen-encoding recombinant molecule; or a combination of a superantigen-encoding recombinant molecule with a cytokine-encoding recombinant molecule and/or a chemokine recombinant molecule of the present invention combined (separately or together) with a delivery vehicle, preferably a liposome, such as disclosed herein.

A therapeutic composition of the present invention, upon entering targeted cells, leads to the production of superantigen, cytokine and/or chemokine protein that activate cytotoxic T cells, natural killer cells, T helper cells and macrophages. Such cellular activation overcomes the otherwise relative lack of immune response to cancer cells, leading to the destruction of such cells.

A therapeutic composition of the present invention is useful for the treatment of cancers, both tumors and metastatic forms of cancer. Treatment with the therapeutic composition overcomes the disadvantages of traditional treatments for metastatic cancers. For example, compositions of the present invention can target dispersed metastatic cancer cells that cannot be treated using surgical methods. In addition, administration of such compositions do not result in the harmful side effects caused by chemotherapy and radiation therapy.

A therapeutic composition of the present invention is preferably used to treat cancers, including, but not limited to, melanomas, squamous cell carcinoma, breast cancers, head and neck carcinomas, thyroid carcinomas, soft tissue sarcomas, bone sarcomas, testicular cancers, prostatic cancers, ovarian cancers, bladder cancers, skin cancers, brain cancers, angiosarcomas, hemangiosarcomas, mast cell tumors, primary hepatic cancers, lung cancers, pancreatic cancers, gastrointestinal cancers, renal cell carcinomas, hematopoietic neoplasias, leukemias and lymphomas. Particularly preferred cancers to treat with a

therapeutic composition of the present invention, include melanomas, lung cancers, thyroid carcinomas, breast cancers, renal cell carcinomas, squamous cell carcinomas, brain tumors and skin cancers. A therapeutic composition of the present invention is useful for treating tumors that can form in such cancers, including malignant and benign tumors.

A therapeutic composition of the present invention is also advantageous for the treatment of infectious diseases as a long term, targeted therapy for primary lesions (e.g., granulomas) resulting from the propagation of a pathogen. As used herein, the term "lesion" refers to a lesion formed by infection of an animal with a pathogen. Preferred therapeutic compositions for use in the treatment of an infectious disease comprise a superantigen-encoding recombinant molecule; or a combination of a superantigen-encoding recombinant molecule, with a cytokine-encoding recombinant molecule and/or a chemokine recombinant molecule of the present invention. More preferred therapeutic compositions for use in the treatment of infectious disease comprise a superantigen-encoding recombinant molecule; or a combination of superantigen-encoding recombinant molecule, with a cytokine-encoding recombinant molecule and/or a chemokine recombinant molecule of the present invention combined with a delivery vehicle, preferably a liposome of the present invention. Similar to the mechanism described for the treatment of cancer, treatment of infectious diseases with superantigen,

cytokine and/or chemokine can result in increased T cell, natural killer cell, and macrophage cell activity that overcome the relative lack of immune response to a lesion formed by a pathogen.

A therapeutic composition of the present invention is particularly useful for the treatment of infectious diseases caused by pathogens, including, but not limited to, intracellular bacteria (i.e., a bacteria that resides in a host cell), internal parasites, pathogenic fungi and endoparasites. Particularly preferred infectious diseases to treat with a therapeutic composition of the present invention include tuberculosis, leprosy, aspergillosis, coccidioidomycosis, cryptococcoses, leishmaniasis and toxoplasmosis.

In order to treat an animal with disease, a therapeutic composition of the present invention is administered to the animal in an effective manner such that the composition is capable of treating that animal from disease. For example, a recombinant molecule, when administered to an animal in an effective manner, is able to stimulate effector cell immunity in a manner that is sufficient to alleviate the disease afflicting the animal. According to the present invention, treatment of a disease refers to alleviating a disease and/or preventing the development of a secondary disease resulting from the occurrence of a primary disease.

An effective administration protocol (i.e., administering a therapeutic composition in an effective

manner) comprises suitable dose parameters and modes of administration that result in treatment of a disease. Effective dose parameters and modes of administration can be determined using methods standard in the art for a particular disease. Such methods include, for example, determination of survival rates, side effects (i.e., toxicity) and progression or regression of disease. In particular, the effectiveness of dose parameters and modes of administration of a therapeutic composition of the present invention when treating cancer can be determined by assessing response rates. Such response rates refer to the percentage of treated patients in a population of patients that respond with either partial or complete remission. Remission can be determined by, for example, measuring tumor size or microscopic examination for the presence of cancer cells in a tissue sample.

In accordance with the present invention, a suitable single dose size is a dose that is capable of treating an animal with disease when administered one or more times over a suitable time period. Doses can vary depending upon the disease being treated. In the treatment of cancer, a suitable single dose can be dependent upon whether the cancer being treated is a primary tumor or a metastatic form of cancer. Doses of a therapeutic composition of the present invention suitable for use with direct injection techniques can be used by one of skill in the art to determine appropriate single dose sizes for systemic administration based on the size of an animal. A suitable

single dose of a therapeutic composition to treat a tumor is a sufficient amount of a superantigen-encoding recombinant molecule; or a superantigen-encoding recombinant molecule, with a cytokine-encoding recombinant molecule and/or a chemokine recombinant molecule to reduce, and preferably eliminate, the tumor following transfection of the recombinant molecules into cells at or near the tumor site. A preferred single dose of the superantigen-encoding recombinant molecule is an amount that, when transfected into a target cell population, leads to the production of from about 250 femtograms (fg) to about 1 μg, preferably from about 500 fg to about 500 picogram (pg), and more preferably from about 1 pg to about 100 pg of superantigen per transfected cell. A preferred single dose of a cytokine-encoding recombinant molecule is an amount that, when transfected into a target cell population, leads to the production of from about 10 pg to about 1 μg, preferably from about 100 pg to about 750 pg, and more preferably about 500 pg of cytokine per transfectant. A preferred single dose of a chemokine-encoding recombinant molecule is an amount that, when transfected into a target cell population, leads to the production of from about 1 fg to about 1 μg, preferably from about 1 pg to about 10 ng, and more preferably from about 1 pg to about 1 ng chemokine per transfectant.

A suitable single dose of a superantigen-encoding recombinant molecule; or a combination of a superantigen-encoding recombinant molecule, with a cytokine-encoding

recombinant molecule and/or a chemokine-encoding recombinant molecule in a non-targeting carrier to administer to an animal to treat a tumor, is an amount capable of reducing, and preferably eliminating, the tumor following transfection of the recombinant molecules into cells at or near the tumor site. A preferred single dose of a therapeutic composition to treat a tumor is from about 100 μg to about 2 milligrams (mg) of total recombinant molecules, more preferably from about 150 μg to about 1 mg of total recombinant molecules, and even more preferably from about 200 μg to about 800 μg of total recombinant molecules. A preferred single dose of a superantigen-encoding recombinant molecule complexed with liposomes, is from about 100 μg of total DNA per 800 nmol of liposome to about 2 mg of total recombinant molecules per 16 micromole (μmol) of liposome, more preferably from about 150 μg per 1.2 μmol of liposome to about 1 mg of total recombinant molecules per 8 μmol of liposome, and even more preferably from about 200 μg per 2 μmol of liposome to about 400 μg of total recombinant molecules per 3.2 μmol of liposome.

A preferred single dose of a cytokine-encoding recombinant molecule or a chemokine-encoding recombinant molecule in a non-targeting carrier to administer to an animal to treat a tumor, is from about 100 μg to about 2 mg of total recombinant molecules, more preferably from about 150 μg to about 1 mg of total recombinant molecules, and even more preferably from about 200 μg to about 400 μg of total recombinant molecules. A preferred single dose of a

cytokine-encoding recombinant molecule or a chemokine-encoding recombinant molecule complexed with liposomes to administer to an animal to treat a tumor, is from about 100 μg of total recombinant molecules per 800 nmol of liposome to about 2 mg of total recombinant molecules per 16 μmol of liposome, more preferably from about 150 μg per 1.2 μmol of liposome to about 1 mg of total recombinant molecules per 8 μmol of liposome, and even more preferably from about 200 μg per 2 μmol of liposome to about 400 μg of total recombinant molecules per 6.4 μmol of liposome.

A preferred single dose of a superantigen-encoding recombinant molecule in a non-targeting carrier to administer to an animal treat a metastatic cancer, is from about 100 μg to about 4 mg of total recombinant molecules, more preferably from about 150 μg to about 3 mg of total recombinant molecules, and even more preferably from about 200 μg to about 2 mg of total recombinant molecules. A preferred single dose of a superantigen-encoding recombinant molecule complexed with liposomes to administer to an animal to treat a metastatic cancer, is from about 100 μg of total recombinant molecules per 800 nmol of liposome to about 4 mg of total recombinant molecules per 32 μmol of liposome, more preferably from about 200 μg per 1.6 μm of liposome to about 3 mg of total recombinant molecules per 24 μmol of liposome, and even more preferably from about 400 μg per 3.2 μmol of liposome to about 2 mg of total recombinant molecules per 16 μmol of liposome.

A preferred single dose of a cytokine-encoding recombinant molecule or a chemokine-encoding recombinant molecule in a non-targeting carrier to administer to an animal to treat a metastatic cancer, is from about 100 μg to about 4.0 mg of total recombinant molecules, more preferably from about 150 μg to about 3 mg of total recombinant molecules, and even more preferably from about 200 μg to about 2 mg of total recombinant molecules. A preferred single dose of a cytokine-encoding recombinant molecule or a chemokine-encoding recombinant molecule complexed with liposomes to administer to an animal to treat a metastatic cancer, is from about 100 μg of total recombinant molecules per 800 nmol of liposome to about 4.0 mg of total recombinant molecules per 32 μmol of liposome, more preferably from about 200 μg per 1.6 μmol of liposome to about 3 mg of total recombinant molecules per 24 μmol of liposome, and even more preferably from about 400 μg per 3.2 μmol of liposome to about 2 μg of total recombinant molecules per 16 μmol of liposome.

According to the present invention, a single dose of a therapeutic composition useful to treat a lesion, comprising a superantigen-encoding recombinant molecule in a non-targeting carrier or liposomes, respectively, and a cytokine-encoding recombinant molecule in a non-targeting carrier or liposomes, respectively, is substantially similar to those doses used to treat a tumor (as described in detail above).

The number of doses administered to an animal is dependent upon the extent of the disease and the response of an individual patient to the treatment. For example, a large tumor may require more doses than a smaller tumor. In some cases, however, a patient having a large tumor may require fewer doses than a patient with a smaller tumor, if the patient with the large tumor responds more favorably to the therapeutic composition than the patient with the smaller tumor. Thus, it is within the scope of the present invention that a suitable number of doses includes any number required to cause regression of a disease. A preferred protocol is monthly administrations of single doses (as described above) for up to about 1 year. A preferred number of doses of a therapeutic composition comprising a superantigen-encoding recombinant molecule; or a combination of a superantigen-encoding recombinant molecule, with a cytokine-encoding recombinant molecule and/or a chemokine-encoding recombinant molecule in a non-targeting carrier or complexed with liposomes in order to treat a tumor is from about 1 to about 10 administrations per patient, preferably from about 2 to about 8 administrations per patient, and even more preferably from about 3 to about 5 administrations per patient. Preferably, such administrations are given once every 2 weeks until signs of remission appear, then once a month until the disease is gone.

A preferred number of doses of a therapeutic composition comprising a superantigen-encoding recombinant

molecule; or a combination of a superantigen-encoding recombinant molecule, with a cytokine-encoding recombinant molecule and/or a chemokine-encoding recombinant molecule in a non-targeting carrier or complexed with liposomes in order to treat a metastatic cancer, is from about 2 to about 10 administrations patient, more preferably from about 3 to about 8 administrations per patient, and even more preferably from about 3 to about 7 administrations per patient. Preferably, such administrations are given once every 2 weeks until signs of remission appear, then once a month until the disease is gone.

According to the present invention, the number of doses of a therapeutic composition to treat a lesion comprising a superantigen-encoding recombinant molecule; or a combination of a superantigen-encoding recombinant molecule, with a cytokine-encoding recombinant molecule and/or a chemokine-encoding recombinant molecule, in a non-targeting carrier or liposomes, respectively, is substantially similar to those number of doses used to treat a tumor (as described in detail above).

A therapeutic composition is administered to an animal in a fashion to enable expression of an introduced recombinant molecule of the present invention into a curative protein in the animal to be treated for disease. A therapeutic composition can be administered to an animal in a variety of methods including, but not limited to, local administration of the composition into a site in an animal. Examples of such sites include lymph nodes, a site

that contains abnormal cells or pathogens to be destroyed (e.g., injection locally within the area of a tumor or a lesion); and systemic administration.

Therapeutic compositions to be delivered by local administration include: (a) recombinant molecules of the present invention in a non-targeting carrier (e.g., as "naked" DNA molecules, such as is taught, for example in Wolff et al., 1990, *Science 247*, 1465-1468); and (b) recombinant molecules of the present invention complexed to a delivery vehicle of the present invention. Suitable delivery vehicles for local administration comprise liposomes. Delivery vehicles for local administration can further comprise ligands for targeting the vehicle to a particular site (as described in detail herein).

A preferred method of local administration is by direct injection. Direct injection techniques are particularly useful for the treatment of disease by, for example, injecting the composition into a mass formed by abnormal cells, a lymph node or a granuloma mass induced by pathogens. Preferably, a recombinant molecule of the present invention complexed with a delivery vehicle is administered by direct injection into or locally within the area of a tumor mass, a lymph node, a granuloma mass or a cancer cell. Administration of a composition locally within an area of a mass or a cell refers to injecting the composition centimeters and preferably, millimeters within the mass or the cell. A preferred tumor mass to inject includes discrete inner body and cutaneous solid tumors.

A preferred inner body tumor to inject includes a discrete solid tumor that forms in the brain, breast, liver, kidney, colon, prostate, testicular, ovary, spleen and/or lymph node. A preferred cutaneous tumor to inject includes a discrete solid melanoma.

A preferred lymph node to inject includes a draining lymph node that "drains" a site containing abnormal cells or pathogens. As used herein, the term "draining lymph node" refers to a lymph node that is located downstream of a site containing abnormal cells or pathogens is based on the direction of the lymphatic flow of an animal (see general discussion in Hole, *Human Anatomy and Physiology*, Edward G. Jaffe, ed., Wm. C Brown Publishers, Dubuque, IA; and G.C. Christiansen et al., *Anatomy of the Dog*, W.B. Saunders Publishers, Philadelphia, PN, 1979; both of which are incorporated herein by this reference). A preferred draining lymph node to inject comprises the draining lymph node most proximal to a site containing abnormal cells or pathogens. Thus, a skilled artisan can choose the site of lymph node injection based upon the location of the site containing abnormal cells or pathogens. Examples of lymph nodes to injection include: the mandibular lymph node if a tumor is located in the oral cavity; and the superficial cervical lymph node of a tumor is located in the front leg region. Effector cells travel from a site containing abnormal cells or pathogens. Injection of a therapeutic composition of the present invention into a lymph node can result in expression of a superantigen, a cytokine and/or

a chemokine by an effector cell from the lymph node or that has drained into the lymph node. Such expression can result in the activation of T lymphocytes, which can travel back to the site containing abnormal cells or pathogens to enhance the immune response at the site.

Another method of local administration is to contact a therapeutic composition of the present invention in or around a surgical wound. For example, a patient can undergo surgery to remove a tumor. Upon removal of the tumor, the therapeutic composition can be coated on the surface of tissue inside the wound or the composition can be injected into areas of tissue inside the wound. Such local administration is useful for treating cancer cells not excised by the surgical procedure, as well as, preventing recurrence of the primary tumor or development of a secondary tumor in the area of the surgery.

In one embodiment, a therapeutic composition of the present invention can be introduced to a tumor cell *in vivo*. In another embodiment, a therapeutic composition of the present invention can be introduced to a non-tumor cell *in vivo* or *in vitro*. Methods to introduce a therapeutic composition *in vivo* are disclosed herein. Methods to introduce a therapeutic composition *in vitro* include methods standard in the art, such as culturing cells in the presence of a therapeutic composition for a sufficient amount of time to enable a nucleic acid molecule of the present invention to pass through the plasma membrane in a cell and subsequently to be expressed in the cell.

Therapeutic compositions useful in systemic administration, include recombinant molecules of the present invention complexed to a targeted delivery vehicle of the present invention. Suitable delivery vehicles for use with systemic administration comprise liposomes comprising ligands for targeting the vehicle to a particular site, preferably ligands for targeting the vehicle to a site of a cancer or a lesion (depending upon the disease being treated). For cancer treatment, ligands capable of selectively binding to a cancer cell or to a cell within the area of a cancer cell are preferred. Systemic administration is useful for the treatment of both tumor and metastatic cancer and systemic infectious diseases. Systemic administration is particularly useful for the treatment of metastatic forms of cancer, in which the cancer cells are dispersed (i.e., not localized within a single tumor mass). Systemic administration is particularly advantageous when organs, in particular difficult to reach organs (e.g., heart, spleen, lung or liver) are the targeted sites of treatment.

Preferred methods of systemic administration, include intravenous injection, aerosol, oral and percutaneous (topical) delivery. Intravenous injections can be performed using methods standard in the art. Aerosol delivery can also be performed using methods standard in the art (see, for example, Stribling et al., *Proc. Natl. Acad. Sci. USA* 189:11277-11281, 1992, which is incorporated herein by reference in its entirety). Oral delivery can be

performed by complexing a therapeutic composition of the present invention to a carrier capable of withstanding degradation by digestive enzymes in the gut of an animal. Examples of such carriers, include plastic capsules or tablets, such as those known in the art. Topical delivery can be performed by mixing a therapeutic composition of the present invention with a lipophilic reagent (e.g., DMSO) that is capable of passing into the skin.

Therapeutic compositions of the present invention can be administered to any animal, preferably to mammals and birds, and more preferably to humans, house pets, economic produce animals and zoo animals. Economic produce animals include animals to be consumed or that produce useful products (e.g., sheep for wool production). Zoo animals include those animals harbored in zoos. Preferred animals to protect include humans, dogs, cats, sheep, cattle, horses and pigs, with humans and dogs being particularly preferred. While a therapeutic composition of the present invention is effective to treat disease in inbred species of animals, the composition is particularly useful for treating outbred species of animals, in particular those having tumors.

Yet another embodiment of the present invention is a method to suppress T cell activity in an animal, the method comprising administering to an animal an effective amount of a therapeutic composition comprising: (a) a naked nucleic acid molecule encoding a superantigen; and (b) a pharmaceutically acceptable carrier, in which the nucleic

acid molecule is operatively linked to a transcription control sequence, and in which the therapeutic composition is targeted to a site in the animal that contains excessive T cell activity.

Suitable embodiments, single dose sizes, number of doses and modes of administration of a therapeutic composition of the present invention useful in a treatment method of the present invention are disclosed in detail herein.

A therapeutic composition of the present invention is also advantageous for the treatment of autoimmune diseases in that the composition suppresses the harmful stimulation of T cells by autoantigens (i.e., a "self", rather than a foreign antigen). Superantigen-encoding recombinant molecules in a therapeutic composition, upon transfection into a cell, produce superantigens that delete harmful populations of T cells involved in an autoimmune disease. A preferred therapeutic composition for use in the treatment of autoimmune disease comprises a superantigen-encoding recombinant molecule of the present invention. A more preferred therapeutic composition for use in the treatment of autoimmune disease comprises a superantigen-encoding recombinant molecule combined with a non-targeting carrier of the present invention, preferably saline or phosphate buffered saline. Such a therapeutic composition of the present invention is particularly useful for the treatment of autoimmune diseases, including but not limited to, multiple sclerosis, systemic lupus

erythematosus, myasthenia gravis, rheumatoid arthritis, insulin dependent diabetes mellitus, psoriasis, polyarteritis, immune mediated vasculitides, immune mediated glomerulonephritis, inflammatory neuropathies and sarcoidosis.

A single dose of a superantigen-encoding nucleic acid molecule in a non-targeting carrier to administer to an animal to treat an autoimmune disease is from about 0.1 μg to about 200 μg of total recombinant molecules per kilogram (kg) of body weight, more preferably from about 0.5 μg to about 150 μg of total recombinant molecules per kg of body weight, and even more preferably from about 1 μg to about 10 μg of total recombinant molecules per kg of body weight.

The number of doses of a superantigen-encoding recombinant molecule in a non-targeting carrier to be administered to an animal to treat an autoimmune disease is an injection about once every 6 months, more preferably about once every 3 months, and even more preferably about once a month.

A preferred method to administer a therapeutic composition of the present invention to treat an autoimmune disease is by local administration, preferably direct injection. Direct injection techniques are particularly important in the treatment of an autoimmune disease. Preferably, a therapeutic composition is injected directly into muscle cells in a patient, which results in prolonged expression (e.g., weeks to months) of a recombinant molecule of the present invention. Preferably, a

recombinant molecule of the present invention in the form of "naked DNA" is administered by direct injection into muscle cells in a patient.

Another aspect of the present invention is an adjuvant for use with a nucleic acid-based vaccine to protect an animal from a disease or a remedy to treat a diseased animal. Adjuvants of the present invention comprise: (a) a superantigen-encoding nucleic acid molecule of the present invention; or (b) a combination of a superantigen-encoding nucleic acid molecule of the present invention with a cytokine nucleic acid molecule of the present invention, a chemokine nucleic acid molecule of the present invention or mixtures thereof.

Suitable compounds to combine with an adjuvant of the present invention, to form an adjuvant composition (i.e., a vaccine composition useful as a preventative therapeutic reagent or a therapeutic remedy useful to alleviate a disease) of the present invention, include any compound that is administered to an animal as an immunogen. As used herein, an immunogen of the present invention comprises a compound capable of eliciting an immune response in an animal. Preferably, an immunogen of the present invention is derived from a foreign agent including a pathogen. Also preferably, an immunogen of the present invention includes an allergen (organic or inorganic), tumor antigens and self-antigens.

A preferred immunogen is derived from a pathogen including, but not limited to, a virus, a bacteria, a

eukaryotic parasite and unicellular protozoa (e.g., amoeba). Preferred eukaryotic parasites include protozoan parasites, helminth parasites (such as nematodes, cestodes, trematodes, ectoparasites and fungi.

A preferred immunogen also includes an allergen including, but not limited to, a plant allergen, an animal allergen, a bacterial allergen, a parasitic allergen, a metal-based allergen that causes contact sensitivity and inorganic allergens such as silica, beryllium, xenobiotics, synthetic drugs and dyes. A more preferred allergen includes weed, grass, tree, peanut, mite, flea, cat, house dust and bacterial products antigens.

A preferred immunogen derived from a bacteria includes an immunogen that protects an animal from or alleviates *Mycobacterium* infection, in particular *M. tuberculosis*, *M. leprae*, *M. avium*, and/or *M. bovis* infection. A more preferred bacterial immunogen of the present invention includes a peptide, mimetopes thereof and compositions containing the same, as disclosed in U.S. Patent Serial No. 08/484,169, filed June 7, 1995, which is incorporated herein by this reference. In one embodiment, an immunogen comprises a nucleic acid molecule encoding an immunogenic protein. Such immunogen-encoding nucleic acid molecules can be designed by those of skill in the art based upon the amino acid sequence of the immunogen. In addition, a recombinant molecule encoding an immunogen of the present invention can be produced using the recombinant DNA technology disclosed herein and known to those of skill

in the art. In other embodiments, an immunogen can comprise a peptide, a polypeptide or a chemical compound as disclosed herein. All such embodiments of an immunogen are useful with an adjuvant of the present invention.

In order to treat an animal (i.e., vaccinate or remedy), an adjuvant composition of the present invention is administered to the animal in an effective manner such that the composition is capable of protecting an animal from or alleviating a disease. For example, an adjuvant, when administered to an animal in an effective manner, is able to stimulate effector cell immunity in a manner that is sufficient to prevent an initial or continued disease response by the subject animal.

An effective administration protocol (i.e., administering an adjuvant composition in an effective manner) comprises suitable dose parameters, and modes and times of administration that result in the treatment of an animal. Effective dose parameters and modes of administration can be determined using methods standard in the art for a particular adjuvant composition. Such methods include, for example: determination of side effects (i.e., toxicity) of an adjuvant composition; progression of a disease upon administration of an adjuvant composition; magnitude and/or duration of antibody response by an animal against an immunogen contained in an adjuvant composition; magnitude and/or duration of a cell mediated immune response in an animal against an adjuvant composition; similarity of an immune response to an adjuvant composition

in different species of animals; and/or effect of breed (in non-human animals) or race (in humans) on responsiveness to an adjuvant composition. In particular, the effectiveness of dose parameters and modes of administration of an adjuvant composition of the present invention can be determined by assessing antibody production *in vivo*, skin test sensitivities *in vivo*, cytokine production *in vitro*, antigen-specific proliferation *in vitro*, cytotoxic T cell activity *in vitro*, reduction of tumor burden *in vivo* and/or reduction of infectious agent burden *in vivo*. Tests standard in the art can be used to determine antibody production (e.g., enzyme-linked immunoassays), skin test sensitivities (e.g., subcutaneous injection of an immunogen into a vaccinated animal to assess weal formation, induration and erythema), cytokine production (e.g., immunoassays using cytokine-specific antibodies or bio-assays using cytokine-dependent cell lines), antigen-specific proliferation (e.g., ^{3}H-thymidine incorporation), cytotoxic T cell activity (e.g., measure release of ^{51}Cr from target cells), reduction of tumor burden (e.g., measure size of a tumor) and/or reduction of infectious agent burden (e.g., obtaining, for example, viral titers, bacterial colony counts or parasite counts).

An effective dose refers to a dose capable of immunizing an animal against an immunogen. Effective doses can vary depending upon, for example, the adjuvant used, the immunogen being administered, and the size and type of the recipient animal. Effective doses to treat an animal

to an immunogen include doses administered over time that are capable of preventing or alleviating a disease in an animal to, for example, a pathogen or allergen. For example, a first treatment dose can comprise an amount of an adjuvant composition of the present invention that causes a minimal hypersensitive response when administered to a hypersensitive animal. A second treatment dose can comprise a greater amount of the same adjuvant composition than the first dose. Effective treatment doses can comprise increasing concentrations of the adjuvant composition necessary to treat an animal such that the animal does not exhibit signs of a disease.

In accordance with the present invention, a suitable single dose is a dose that is capable of vaccinating an animal against a foreign agent when administered one or more times over a suitable time period. For example, a preferred single dose of an adjuvant composition of the present invention is from about 100 μg to about 1 mg of the adjuvant composition per kilogram body weight of the animal. Further treatments with the adjuvant composition can be administered from about 1 week to about 1 year after the original administration. Further treatments with the adjuvant composition preferably are administered when the animal is no longer protected from an immunogen to which the animal has been treated. Particular administration doses and schedules can be developed by one of skill in the art based upon the parameters discussed above.

The number of doses administered to an animal is dependent upon the immunogen and the response of an individual patient to the adjuvant composition. For example, treatment of one strain of virus may require more doses than treatment of a more immunogenic strain of virus. Thus, it is within the scope of the present invention that a suitable number of doses includes any number required to treat an animal. A preferred number of doses of an adjuvant composition comprising a superantigen-encoding recombinant molecule, and/or a cytokine-encoding recombinant molecule and/or a chemokine-encoding recombinant molecule is from about 2 to about 20 administrations, preferably from about 3 to about 10 administrations, and even more preferably from about 3 to about 5 administrations per patient per year. Preferably, such administrations are given once every 2 weeks until, for example, antibody production against an immunogen increases or decreases, cell mediated immunity increases, and/or a clinical response is observed when an adjuvant composition is administered as a therapeutic remedy.

A preferred single dose of the superantigen-encoding recombinant molecule is an amount that, when transfected into a muscle cells, skin tissue, lung cells or other suitable cellular sites, leads to the production of from about 10 femtograms (fg) to about .01 μg, preferably from about 100 fg to about 1 picogram (pg), and more preferably from about 1 pg to about 5 pg of superantigen per transfected cell. A preferred single dose of a cytokine-

encoding recombinant molecule is an amount that when transfected into a target cell population leads to the production of from about 10 pg to about .01 μg, preferably from about 100 fg to about 2 pg, and more preferably about 1 pg of cytokine per transfected. A preferred single dose of a chemokine-encoding recombinant molecule is an amount that when transfected into a target cell population leads to the production of from about 1 pg to about .01 μg, preferably from about 0.1 pg to about 10 pg, and more preferably about 1 pg of chemokine per transfected.

In one embodiment, an adjuvant composition of the present invention comprises up to about 50% of an immunogen-encoding recombinant molecule and up to about 50% of a superantigen-encoding recombinant molecule. Preferably, an adjuvant composition of the present invention comprises no more than about 1.5 mg of immunogen-encoding recombinant molecule and no more than about 1.5 mg of superantigen-encoding recombinant molecule, more preferably no more than about 1 mg of immunogen-encoding recombinant molecule and no more than about 1 mg of superantigen-encoding recombinant molecule, and even more preferably no more than about 0.5 mg of immunogen-encoding recombinant molecule and no more than about 0.5 mg of superantigen-encoding recombinant molecule per animal.

In another embodiment, an adjuvant composition of the present invention comprises an immunogen-encoding recombinant molecule in an amount up to about 66% by weight of the composition and a superantigen-encoding recombinant

molecule in an amount up to about 33% by weight of the composition. Preferably, an adjuvant composition of the present invention comprises no more than about 2000 μg of immunogen-encoding recombinant molecule and no more than about 1000 μg of superantigen-encoding recombinant molecule, more preferably no more than about 1400 μg of immunogen-encoding recombinant molecule and no more than about 660 μg of superantigen-encoding recombinant molecule, and even more preferably no more than about 670 μg of immunogen-encoding recombinant molecule and no more than about 330 μg of superantigen-encoding recombinant molecule per animal.

In another embodiment, an adjuvant composition of the present invention comprises an immunogen-encoding recombinant molecule in an amount up to about 50% of the composition; a superantigen-encoding recombinant molecule in an amount up to about 25% of the composition; and a cytokine-encoding recombinant molecule or chemokine-encoding recombinant molecule or mixtures thereof, in an amount up to about 25% of the composition. According to the present embodiment, a cytokine-encoding recombinant molecule or a chemokine-encoding recombinant molecule can be used alone or in combination with each other. When used in combination, the ratio of cytokine-encoding recombinant molecule to chemokine-encoding recombinant molecule can be varied according to need. The ratio can be determined based upon the effectiveness of the adjuvant composition at

vaccinating an animal against a foreign agent using the methods and parameters disclosed herein.

In one embodiment, an adjuvant composition of the present invention comprises: no more than about 2000 μg of immunogen-encoding recombinant molecule, no more than about 500 μg of superantigen-encoding recombinant molecule, and no more than about 500 μg of cytokine-encoding recombinant molecule or no more than about 500 μg of chemokine-encoding recombinant molecule; more preferably no more than about 1400 μg of immunogen-encoding recombinant molecule, no more than about 300 μg of superantigen-encoding recombinant molecule, and no more than about 300 μg of cytokine-encoding recombinant molecule or no more than about 300 μg of chemokine-encoding recombinant molecule; and even more preferably no more than about 660 μg of immunogen-encoding recombinant molecule, no more than about 160 μg of superantigen-encoding recombinant molecule, and no more than about 160 μg of cytokine-encoding recombinant molecule or no more than about 160 μg of chemokine-encoding recombinant molecule per animal.

In another embodiment, an adjuvant composition of the present invention comprises: no more than about 2000 μg of immunogen-encoding recombinant molecule, no more than about 500 μg of superantigen-encoding recombinant molecule, and no more than about 250 μg of cytokine-encoding recombinant molecule and no more than about 250 μg of chemokine-encoding recombinant molecule; more preferably no more than about 1000 μg of immunogen-encoding recombinant molecule,

no more than about 250 μg of superantigen-encoding recombinant molecule, and no more than about 125 μg of cytokine-encoding recombinant molecule and no more than about 125 μg of chemokine-encoding recombinant molecule; and even more preferably no more than about 660 μg of immunogen-encoding recombinant molecule, no more than about 160 μg of superantigen-encoding recombinant molecule, and no more than about 80 μg of cytokine-encoding recombinant molecule and no more than about 80 μg of chemokine-encoding recombinant molecule per animal.

Adjuvant compositions are preferably delivered by intramuscular administration in the form of "naked" DNA molecules, such as disclosed herein. Preferably, an adjuvant composition of the present invention is delivered by intramuscular, intravenous, intraperitoneal and/or intraarterial injection, and/or injection directly into specific cellular locations (e.g., into a tumor). Preferred sites of intramuscular injections include caudal thigh muscle, back muscle and into the buttocks of a human.

Preferably, an adjuvant composition of the present invention comprises a suitable pharmaceutically acceptable carrier for delivering the composition intramuscularly. A preferred carrier for use with an adjuvant includes phosphate buffered saline, water, Ringer's solution, dextrose solution, Hank's balanced salt solution and normal saline. A more preferred carrier includes phosphate

buffered saline and normal saline, with phosphate buffered saline being even more preferred.

Preferably, an adjuvant composition of the present invention comprises a mixture including a superantigen encoding molecule including an SEA-encoding recombinant molecule, an SEB-encoding recombinant molecule or mixtures thereof, and an immunogen-encoding recombinant molecule of the present invention; a superantigen encoding molecule including an SEA-encoding recombinant molecule, an SEB-encoding recombinant molecule or mixtures thereof, a cytokine encoding molecule including a GM-CSF-encoding recombinant molecule and an immunogen-encoding recombinant molecule of the present invention; or a superantigen encoding molecule including an SEA-encoding recombinant molecule, an SEB-encoding recombinant molecule or mixtures thereof, a chemokine encoding molecule including a MIP1α, MIP1β, IL-8 or RANTES recombinant molecule and an immunogen-encoding recombinant molecule of the present invention.

In a preferred embodiment, an adjuvant of the present invention includes the following recombinant molecules contained in phosphate buffered saline: (1) PCR_3-SEA, PCR_3-SEA.S, PCR_3-SEB, PCR_3-SEB.S, PCR_3-TSST and mixtures thereof; (2) mixtures of up to about 50% PCR_3-SEA, PCR_3-SEA.S, PCR_3-SEB, PCR_3-SEB.S and/or PCR_3-TSST, and up to about 50% PCR_3-GM_3; (3)

mixtures of up to about 50% PCR_3-SEA, PCR_3-SEA.S, PCR_3-SEB, PCR_3-SEB.S and/or PCR_3-TSST, and up to about 50% PCR_3-MIP1α;

(4) mixtures of up to about 50% PCR_3-SEA, PCR_3-SEA.S, PCR_3-SEB, PCR_3-SEB.S and/or PCR_3-TSST, and up to about 50% PCR_3-MIP1β; (5) mixtures of up to about 50% PCR_3-SEA, PCR_3-SEA.S, PCR_3-SEB, PCR_3-SEB.S and/or PCR_3-TSST, and up to about 50% PCR_3-RANTES; (6) mixtures of up to about 50% PCR_3-SEA, PCR_3-SEA.S, PCR_3-SEB, PCR_3-SEB.S and/or PCR_3-TSST, up to about 25% PCR_3-GM_3, and up to about 25% PCR_3-MIP1α, PCR_3-MIP1β and/or PCR_3-RANTES.

According to the present invention, a preferred embodiment of an adjuvant composition of the present invention includes: (1) an immunogen-encoding recombinant molecule the present invention in an amount up to about 50% of the composition and a preferred embodiment of an adjuvant of the present invention in an amount up to about 50% of the composition; or (2) an immunogen-encoding recombinant molecule in an amount up to about 66% of the composition and a preferred embodiment of an adjuvant of the present invention in an amount up to about 33% of the composition, in phosphate buffered saline.

The following examples are provided for the purposes of illustration and are not intended to limit the scope of the present invention.

EXAMPLES

Example 1

This example describes the production of recombinant molecules encoding superantigens and cytokines.

Full-length cDNA encoding *Staphylococcal enterotoxin* B (SEB; SEQ ID NO:1) and *Staphylococcal enterotoxin* A (SEA;

SEQ ID NO:3) were produced by polymerase chain reaction (PCR) amplification using templates obtained from Dr. John Kappler (National Jewish Center for Immunology and Respiratory Disease, Denver, CO). A truncated form of SEB lacking the leader sequence, which spans base pairs 46 to 773 (referred to herein as SEB.S), was prepared by PCR amplification using the primers 5' GGGAATTCCATGGAGAGTCAACCAG 3' (SEQ ID NO:7) and 3' GCGGATCCTCACTTTTTCTTTGT 5' (SEQ ID NO:8). A truncated form of SEA lacking the signal sequence, which spans base pairs 46 to 751 (referred to herein as SEA.S), was prepared by PCR amplification using the primers 5' GGGAATTCCATGGAGAGTCAACCAG 3' (SEQ ID NO:9) and 5' GCAAGCTTAACTTGTATATAAATAG 3'(SEQ ID NO:10). Full-length cDNA encoding Toxic Shock Syndrome Toxin (TSST; SEQ ID NO:5) was produced by PCR amplification using a template obtained from Dr. Brian Kotzin (National Jewish Center for Immunology and Respiratory Disease, Denver, CO), using the primers:

5' CGGGGTACCCCGAAGGAGGAAAAAAAAATGTCTACAAACGATAATATAAAG 3' (SEQ ID NO:11); and

3' TGCTCTAGAGCATTAATTAATTTCTGCTTCTATAGTTTTTAT 5' (SEQ ID NO:12).

Each cDNA clone was ligated into the eukaryotic expression vector PCR_3 (In Vitrogen, San Diego, CA) using standard cloning methods. The full-length SEB cDNA cloned into PCR_3 is referred to herein as PCR_3-SEB; the full-length SEA cDNA cloned into PCR_3 is referred to herein as PCR_3-SEA;

the full-length TSST cDNA cloned into PCR_3 is referred to herein as PCR_3-TSST; the truncated SEB cDNA cloned into PCR_3 is referred to herein as PCR_3-SEB.S; and the truncated SEA cDNA cloned into PCR_3 is referred to herein as PCR_3-SEA.S.

A cDNA for canine GM-CSF was produced by PCR amplification of total RNA extracted from Concavalin A-stimulated normal canine peripheral blood mononuclear cells (PBMC) using canine GM-CSF primers designed based on the published canine GM-CSF cDNA (Nash, *ibid.*). The total RNA was reverse transcribed using the reverse transcriptase enzyme and oligoT primers. The canine GM-CSF cDNA was then amplified using PCR and specific 5′ and 3′ primers. The PCR product was cloned into the PCR_3 vector, the resulting recombinant molecule is referred to herein as PCR_3-GM_3.

Example 2

This example describes the expression of DNA encoding superantigens in mammalian CHO cells following transfection.

Isolated PCR_3-SEB.S, PCR_3-SEA.S and PCR_3-TSST were transformed into *E. coli* cells and ampicillin-resistant bacterial colonies were screened for the presence of the plasmid. Selected colonies were then cultured in large scale culture (liter volume). Plasmid DNA was isolated using standard methods. A typical plasmid yield was 20 mg plasmid DNA from one liter of bacteria-containing culture medium. Plasmid DNA was transfected into Chinese hamster ovary cells (CHO) by lipofection (Lipofectamine, Gibco-BRL, Gaithersburg, MD) using methods provided by the

manufacturer. About 2.0 μg of each plasmid DNA was separately transfected into about 10^6 CHO cells.

The transfected CHO cells were cultured for 48 hours. Supernatants and cell lysates were then isolated to determine the amount of intracellular and secreted SAg protein produced by the transfected cells. Cell lysates were prepared by detaching and sonicating the transfected cells to prepare cell lysates to measure activity. SAg protein activity in each sample was measured by quantitating the ability of the SAg protein to stimulate lymphocyte contained in a PBMC population using the following method. Supernatants and lysates to be tested were added in serial dilutions to triplicate wells of a 96-well plate containing 5 X 10^5 PBMC in a total volume of 200 μl per well. After 3 days, the wells were pulsed with ^3H thymidine and incubated for 18 hours. The radioactivity incorporated into the PBMC's were quantitated on a beta counter. Negative controls included CHO cells transfected with the DNA vector without an inserted gene (mock) and positive controls were purified recombinant SAg proteins.

The results were plotted as the mean incorporated thymidine in counts per minute and are shown in Fig. 1. The results indicate that both supernatants and lysates of CHO cells transfected with PCR_3-SEB.S, PCR_3-SEA.S and PCR_3-TSST stimulated strong proliferation of the PBMC's, compared to mock transfected cultures. The activity in supernatants in some cases exceeded that in cell lysates.

Thus, DNA encoding bacterial SAg proteins are capable of being transcribed and translated in mammalian cells in biologically active form. The results also indicate that the amounts of biologically active SAg protein are active produced by the transfected cells was sufficient to stimulate T cell proliferation.

Example 3

This example describes the expression of DNA encoding superantigens in canine melanoma cells following transfection.

A melanoma cell line was established from an oral malignant melanoma obtained by biopsy from a canine patient by isolating a portion of a melanoma tumor, digesting that portion with collagenase and plating the released cells in 24 well plates using Iscove Modified Dulbecco's Medium (IMDM) with 10% fetal calf serum. Melanoma cells were transfected with PCR_3-SEB.S, PCR_3-SEA.S and PCR_3-TSST by lipofection as described in Example 2. The cells were then irradiated (15,000 Rads). Four samples of each sample of transfected melanoma cells were prepared, in which decreasing numbers of the transfected cells were added to normal canine PBMC (5×10^5/well). Each sample was prepared in triplicate in a 96 well plate. After 3 days, proliferation was quantitated as described in Example 2. Non-transfected melanoma cells were used as negative control samples. The results were plotted as the mean incorporated thymidine in counts per minute and are shown in Fig. 2. The results indicate that Canine PBMC

proliferated when cultured with canine melanoma cells transfected with PCR_3-SEB.S, PCR_3-SEA.S and PCR_3-TSST, exhibiting a dose-dependent increase in proliferation as increasing numbers of irradiated tumor cells were used. Thus, melanoma tumor cells can be transfected and can express biologically active SAg protein. The results also show that the transfected melanoma cells continue to release biologically active SAg protein after irradiation, indicating that transfected tumor cells would also be useful as an autologous tumor vaccine as described in detail in the present application.

Example 4

This example describes the long term expression of DNA encoding SEB.S and SEA.S in stably transfected CHO cells.

To determine whether the SAg protein activity detected in supernatants of transfected CHO cells (described in Example 2) represented actual secretion or simple release from dying cells, stably transfected CHO lines were prepared using either PCR_3-SEB.S, PCR_3-SEA.S or vector with no cDNA insert (control). About 2 x 10^6 CHO cells were transfected with about 2 μg of plasmid DNA by lipofection. The transfected cells were then cultured in G418 (1 mg/ml) for 3 weeks to obtain stable transfectants. The G418 selected CHO cells were seeded into 9 individual tissue culture wells, allowed to adhere for 4 hours, and then fresh tissue culture media was added. Supernatants were harvested sequentially, beginning at time zero and continuing for 36 hours. Supernatants were added to PBMC

to assay for SAg protein activity, as described in Example 2.

The results were plotted as the mean proliferation stimulating activity contained in supernatants at each time point and are shown in Figs. 3A and 3B. The results indicate that a steady time-dependent increase in PBMC stimulatory activity was observed in supernatants from CHO cells stably transfected with both PCR_3-SEB.S and PCR_3-SEA.S. Thus, transfection of mammalian cells with PCR_3-SEB.S, PCR_3-SEA.S results in long term expression of biologically active SAg protein. The data indicates that transfected mammalian cells can serve as a sustained source of SAg protein production.

Example 5

This example describes that transfection of PCR_3-SEA.S DNA in melanoma cells results in the expression of biologically active SEA.S protein.

Superantigens are capable of stimulating the proliferation of T cells bearing certain Vβ domains in their T cell receptor (TCR). SEA is known to stimulate T cells having a Vβ3+ TCR in mice. SEB does not stimulate Vβ3+ T cells. Therefore, an experiment was performed to assess the ability of SEA.S protein expressed by melanoma cells transfected with PCR_3-SEA.S DNA to stimulate the proliferation of a T cell clone (AD10) expressing the Vβ3+TCR.

B16 melanoma cells were transfected with PCR_3-SEA.S DNA, PCR_3-SEB.S or PCR_3 vector DNA with no insert (mock).

The cells were then irradiated (18,000 Rads) and plated in triplicate in a 96 well plate at a concentration of about 1 x 10^4 per well. About 1 x 10^5 AD10 cells were added to each well. Next, irradiated syngeneic spleen cells were added to each well as a source of antigen presenting cells for the superantigen and T cell interaction. Negative controls included mock transfected cells; positive controls included recombinant SEA (10ng/ml). The cells were incubated for 48 hours. ^3H thymidine was then added to each well and the proliferative response quantitated.

The results were plotted as the mean incorporated thymidine in counts per minute and are shown in Fig. 4. The AD10 cells proliferated strongly in response to SEA.S protein produced by the PCR_3-SEA.S DNA transfected into the B16 cells, with the proliferative response nearly equal to that of the recombinant protein. Thus, the T cell response generated by transfection of melanoma cells with PCR_3-SEA.S DNA is specific for the correct TCR. Cells transfected with PCR_3-SEB.S DNA did not stimulate proliferation of AD10 cells, consistent with the predicted TCR specificity of SEA and SEB.

Example 6

This example describes the expression of PCR_3-GM DNA in CHO cells.

PCR_3-GM DNA was produced, isolated and transfected into CHO cells using the methods described in Examples 1 and 2. Expression of GM-CSF protein in the CHO cells was measured by the following method. Supernatants were isolated from

the cultures of the transfected cells and non-transfected CHO cells. The supernatants were added to cultures of monocyte cells (obtained from normal canine PBMC) and the ability of the supernatants to support the growth and survival of monocytes was determined. After 4 days in culture with test or control CHO supernatants, monocyte survival was quantitated by addition of methyltetrazolium dye (MTT) that is reduced in viable cells. Absorbance of light at 570 nm (measured using an ELISA reader) is representative of cell survival.

The results are shown in Fig. 5 and indicate that the supernatants from CHO transfected with PCR_3-GM DNA stimulated the survival of canine monocytes in culture compared with results obtained using the control supernatants. The level of activity was comparable to that of 1 x 10^5 units of canine recombinant GM-CSF. Thus, the GM-CSF protein produced by CHO cells transfected with PCR_3-GM DNA is biologically active.

Example 7

This example demonstrates that the vaccination of mice with autologous tumor cells transfected with PCR_3-SEA.S DNA or PCR_3-SEB.S DNA induce strong cytotoxic T cell (CTL) activity.

The following experiment studies the ability of non-immunogenic murine melanoma cells (B16 melanoma cells, F10 clone) expressing either PCR_3-SEA.S DNA or PCR_3-SEB.S to induce CTL responses in mice. B16 cells are known to be non-immunogenic when injected into C57B16/J mice. The

level of CTL responses that can be induced has been shown to correlate with the ability of the immunized animal to reject tumors.

B16 cells were transfected with either PCR_3-SEA.S DNA, PCR_3-SEB.S or PCR_3 DNA lacking insert (mock) using the method described in Example 2. The cells were then irradiated at 12,000 Rads. About 10^6 irradiated cells were then injected subcutaneously into C57B16/J mice. Three weeks later, the mice were sacrificed and their spleen mononuclear cells harvested. Mononuclear cells isolated from the spleen cells were then restimulated *in vitro* with irradiated, non-transfected wild type B16 cells for 6 days in media with interleukin-2 (IL-2). The spleen cells were then added in decreasing numbers to about 5 x 10^3 of ^{51}Cr-labeled wild type (non-transfected) B16 cells in a standard chromium release assay for CTL activity. After 4 hours, the supernatants were harvested and the percentage of specific lysis of the target B16 melanoma cells was quantitated.

The results are shown in Figs. 6A and 6B and indicate that injection of animals with irradiated transfected melanoma cells induce greater CTL activity than injection with non-transfected cells. This result is consistent with the non-immunogenic nature of B16 cells. Thus, DNA encoding bacterial SAg proteins expressed in transfected tumor cells are capable of eliciting strong CTL-mediated immunity against the non-transfected parental cell. These results suggest that autologous tumor cells transfected

with DNA encoding a superantigen constitute an effective tumor vaccine for treatment or prevention of metastatic disease.

Example 8

This example demonstrates that tumor cells transfected with PCR_3-SEB.S DNA are capable of inducing cytotoxic activity in adjacent T cells.

T cells were prepared from a mouse immunized with non-transfected B16 cells using the methods described in Example 7. These isolated cells exhibited minimal CTL activity towards non-transfected B16 target cells. B16 cells were transfected with PCR_3-SEB.S using the methods generally described in Example 2. Induction of CTL activity by the transfected B16 target cells was assessed in a standard 4 hour chromium release assay as used in Example 7.

The results are shown in Fig. 7 and indicates that B16 cells transfected with PCR_3-SEB.S produced protein that rapidly induced a four-fold increase in CTL activity in T cells that were relatively unresponsive to non-transfected target B16 cells. Thus, the SEB produced in the vicinity of the isolated T cells by the B16 cells is capable of stimulating such T cells. The data indicates that tumor cells transfected *in vivo* with PCR_3-SEB.S can produce biologically active SEB.S that is capable of rapidly activating T lymphocytes in their vicinity and thereby inducing cytotoxic activity against themselves or neighboring tumor cells.

Example 9

This example describes the treatment of canine melanoma with DNA encoding superantigen or GM-CSF.

A. Criteria for entry and trial design

Animals selected for entry into the present study were client owned animals with spontaneous oral malignant melanoma, a highly malignant neoplasm of dogs for which there is no alternative effective treatment. Prior to entry, the owners were required to sign informed consent. The study consisted of an initial 12 week trial response phase with 6 injections given once every 2 weeks, followed by long term once monthly maintenance therapy for those animals that responded during the initial 12 week induction phase. Potential toxicity was assessed by (1) body temperature measured daily for 7 days after injection; (2) physical examination of the injection site; (3) owner's assessment of their pet's attitude and appetite; (4) complete blood counts and biochemistry measurements once monthly. Treatment responses were assessed by: (1) physical measurement of tumor dimensions; (2) tumor photography; (3) thoracic radiographs for metastasis evaluation.

B. Superantigen + GM-CSF Treatment protocol

DNA samples complexed with liposomes were prepared as follows. PCR_3-SEB.S and PCR_3-GM plasmid DNA prepared from bacterial cultures by the alkaline lysis method, then purified by CsCl banding, were resuspended at a 1.0 mg/ml concentration in sterile PBS. Liposomes were prepared by

mixing equimolar amounts of N-[1-(33-dioleyloxy)propyl]-N,N,N-triethylammonium (DOTMA; obtained from Syntex, Corp., Palo Alto, CA) and dioleoyl phosphatidylethanolamine (DOPE; obtained from Avanti Polar Lipids, Birmingham, AL). The lipids were dried in a desiccator and reconstituted at a concentration of 1.0 mg/ml in sterile phosphate buffered saline (PBS), pH 7.0. The reconstituted lipids were sonicated for 5 minutes to produce liposomes having an average size of about 200 nm to about 400 nm.

Thirty minutes prior to injection into the patients, the PCR_3-SEB.S and PCR_3-GM DNA was mixed with the liposomes at a ratio of 1.0 μg DNA to 4 nmol liposome, in 1.0 ml sterile PBS. The solution was allowed to complex at room temperature. Two doses of DNA were administered, depending on tumor volume. For tumors less than 3 centimeters (cm) in diameter, 400 μg total DNA (200 μg each of PCR_3-SEB.S and PCR_3-GM DNA) were injected into each tumor. For tumors larger than 3 cm diameter, a total of 800 μg DNA (400 μg each of PCR_3-SEB.S and PCR_3-GM DNA) were injected into each tumor.

For each treatment, the DNA:liposome mixture was injected into the tumor site with a 3 ml syringe and 25 gauge needle. For larger tumors, most of the injection was delivered into tissues at the periphery of the tumor base. For some smaller tumors, injections were also injected directly into tumor tissue. Lymph node tissue having evidence of tumor metastasis was also injected. Injections were performed once every 2 weeks for the first 12 weeks,

then continued twice monthly for those animals in which an initial treatment response occurred, until complete tumor regression occurred. At that time, the frequency of injections decreased to once monthly. The toxicity of the treatment was evaluated based on the parameters outlined above in section A. The results are shown below in Table 1.

Table 1. Patient Log for SEB.S and PCR_3-GM DNA Treatment of Canine Melanoma

Patient	Stage	TN	Tumor Size	Start Date	Response	Comments
Zomax	I	T1bNOMO	1.5 cm diam	5/16/94	CR 51 wks	SEB.S + GM-CSF
Shadow	III	T2bN1bMO	3 cm diam	5/23/94	CR 50 wks	SEB.S + GM-CSF
NG	I	T1NOMO	1.2 cm diam	9/12/94	CR 34 wks	SEB.S + GM-CSF
Maggie	II	T2aNOMO	2 cm diam	8/24/94	PR 33 wks	SEB.S + GM-CSF
K.C.	III	T3aNOMO	> 4 cm diam	10/13/94	SD 12 wk	SEB.S + GM-CSF
Belvedere	III	T2N1bMO	4 cm diam	10/13/94	CR 30 wks	SEB.S + GM-CSF
Nicholas	III	T3bNOMO	> 4 cm diam	2/15/95	SD 12 wks	SEB.S + GM-CSF
Heidi	III	TON1bMO	LN:2cm diam	2/27/95	PR 10 wks	SEB.S + GM-CSF
Bear	III	TON1bMO	LN:2.5cm	4/11/95	SD 4 wks	SEB.S + GM-CSF

Key to terminology in patient data sheets:

Stage: I represents the smallest and III the largest size, with metastases
TNM: World Health Organization staging system
SD = stable disease (no tumor growth)
PR = partial remission (> 50% decrease in tumor size)
CR = tumor completely regressed
PD = progressive disease, no response to treatment
MCT = mast cell tumor
Mammary CA = mammary gland adenocarcinoma (malignant breast cancer)
Thyroid CA = thyroid adenocarcinoma
SCC = squamous cell carcinoma

The results shown in Table 1 indicate that a treatment response was observed in 6 of 9 dogs treated for the 12 week trial period. This included 4 complete remissions (no residual tumor) and 2 partial remissions (greater than 50% reduction in tumor size). Tumors in the remaining two dogs did not regress, but also did not progress in size during the 12 week trial. On average, a tumor response required 6 to 10 weeks to become apparent. The injections did not cause any inflammation or necrosis at injections sites. Toxicity, either local or systemic, was not observed in any of the 10 patents treated in this study. These results provide evidence of the efficacy of direct DNA injection using DNA encoding superantigen (SEB) and cytokine (GM-CSF) for treatment of spontaneous malignant melanoma in an outbred species.

Canine melanoma is a highly malignant, rapidly growing tumor of dogs, and provides a useful model for the study of treatments for human melanoma. Without treatment, the 50% survival time for animals with stage III disease (5 of the patients in this study) is about 3 months and all animals will be dead by 5 months due to pulmonary metastases. The observation of several long term survivors shown in Table 1 (others have not been treated long enough to evaluate) suggests that the combined DNA immunotherapy approach also has a systemic effect on preventing metastatic diseases.

Another major advantage of this approach is the apparent complete absence of toxicity in the dogs. Since dogs respond to SAg protein similar to humans, it is also

likely that toxicity in humans would also be minimal. The delivery of DNA encoding superantigens into tumor cells by transfection and subsequent local expression is sufficient to induce a strong immune response without inducing toxicity. Thus this genetic approach to tumor immunotherapy offers advantages over conventional chemotherapy and radiation therapy in terms of reducing patient morbidity. In addition, delivering the SAg protein by DNA transfection also avoids the potential toxicity associated with systemic administration.

C. Single Gene Treatment Protocol

To evaluate the effectiveness of injecting DNA encoding either a superantigen or a cytokine, relative to combined genetic therapy (SAg-encoding DNA and cytokine-encoding DNA), 2 groups of dogs were treated with either PCR_3-SEB.S DNA alone (3 dogs) or PCR_3-GM DNA alone (3 dogs; 2 entered, one evaluatable). Similar criteria for entry and trial design described above in Section A of this example was applied. Although not formally randomized, after the first 10 dogs were treated with the 2 gene combination, the next 3 enrollees were assigned the PCR_3-SEB.S DNA alone group and the next 3 to the PCR_3-GM DNA alone group. A similar treatment protocol as described above in section B was applied in this study. Briefly, the DNA was complexed with liposomes and injected once every 2 weeks for the first 12 weeks, then continued twice monthly for those animals in which an initial treatment response occurred, until complete tumor regression occurred. The

toxicity of the treatment was evaluated based on the parameters outlined above in section A. The results are shown below in Table 2.

Table 2. Patient Log for SEB.S or PCR_3-GM DNA alone Treatment of Canine Melanoma

Patient	Stage	TN	Tumor Size	Start Date	Response	Comments
Jessie	II	T2bNOMO	2 cm diam	1/11/95	PD 17 wks	SEB.S alone
Mr. T	III	TON1bMO	LN:2cm diam	2/1/95	PD 14 wks	SEB.S alone
Duffy	II	T2aNOMO	2 cm diam	2/3/95	PD 12 wks	SEB.S alone
Scooter	I	T2aNOMO	2 cm diam	3/24/95	PD 7 wks	GM-CSF alone

The results indicated that a tumor response did not occur in any dog receiving PCR_3-SEB.S DNA alone and tumors grew progressively. In addition, one dog (Scooter) treated with PCR_3-GM DNA alone also exhibited progressive growth. These data indicate that treatment with PCR_3-SEB.S DNA alone or PCR_3-GM DNA alone does not induce tumor regression. The data indicate that the marked anti-tumor efficacy of direct DNA injection results from the combined expression of PCR_3-SEB.S DNA and PCR_3-GM DNA in a tumor and adjacent tissues.

Example 10

This example describes the treatment of various tumor types with superantigen or GM-CSF encoding DNA.

The efficacy and lack of toxicity of PCR_3-SEB.S DNA and PCR_3-GM DNA was determined for the treatment of dogs with malignancies having similar biological and histological characteristics as human cancers. Dogs with five different cancers (advanced mammary carcinoma, mast cell tumor, thyroid carcinoma, non-oral melanoma, and squamous cell carcinoma) were treated in this study. Animals selected for entry into the present study included dogs with spontaneous malignancies that had received alternative treatments (e.g., chemotherapy and/or surgery) and either, had not responded, or had relapsed.

Therapeutic samples were prepared and injected intratumorally with PCR_3-SEB.S DNA and PCR_3-GM DNA as described above in Example 2. The dogs were treated initially once every 2 weeks for 12 weeks, then continued twice monthly for those animals in which an initial

treatment response occurred. The toxicity of the treatment was evaluated based on the parameters outlined above in Example 9, section A. The results are shown below in Table 3.

Table 3. Patient Log for SEB.S and PCR_3-GM DNA Treatment of Various Carcinomas

Patient	Tumor Type	Stage	TN	Tumor Size	Start Date	Response	Comments
Emma	Mammary CA	III	T4N1bNMO	1.8 cm diam	8/11/94	PR 22 wks	SEB.S + GM-CSF
Baby	Mammary CA	II	T1aN1bMO	2.6 cm diam	9/12/94	PR 8 wks	SEB.S + GM-CSF
Christa	MCT	IIIa	NA	>2 cm diam	7/27/94	SD 39 wks	SEB.S + GM-CSF
Jack	MCT	IIIa	NA	>3 cm diam	3/28/95	PD 4 wks	SEB.S + GM-CSF
Britt	Thyroid CA	III	T3bNOMO	>7 cm diam	10/14/94	SD 16 wk	SEB.S + GM-CSF
Duncan	Melanoma Toe	NA*	T2N1MO	>4 cm diam	8/11/94	SD 20 wks	SEB.S + GM-CSF
Billy	Melanoma Toe	NA*	TON1bMO	LN 3.5 cm	1/10/95	CR 17 wks	SEB.S + GM-CSF
Scotche	SCC Tonsil	NA	T3NOMO	4 cm diam	3/27/95	SD	SEB.S + GM-CSF

* Metastases
NA Not Applicable
CA Carcinoma
MCT Mast Cell Tumor
SCC Squamous Cell Carcinoma

In this study, toxicity was not observed in any of the animals. Tumor responses (partial remission of the primary tumors) were observed in the animals with mammary carcinoma and neither animal developed additional metastatic disease during the course of the study. Treatment of one dog (Billy) with a large, metastatic (lymph node metastases), non-oral melanoma resulted in complete remission of the cancer. Treatment of the other dog (Duncan) with a large, metastatic (lymph node metastases), non-oral melanoma resulted in prolonged stabilization of the disease. The dog with thyroid cancer (Britt) also experienced prolonged stabilization of the disease with once monthly injections. The response rate for the dogs with mast cell tumors was low. The effectiveness of the treatment on the squamous cell carcinoma is in early stages of evaluation. Taken together, the results indicate that PCR_3-SEB.S DNA and PCR_3-GM DNA can effectively treat multiple tumor types, in addition to the melanomas reported above in Example 9.

Example 11

This example describes the injection of PCR_3-SEA.S DNA into muscle cells which induces potent, long-lasting T cell deletion.

Four groups of mice B10.BR (2-3 mice per group) were prepared as follows. Group (1) consisted of untreated mice (control mice). Group (2) consisted of mice injected intraperitoneally with 100 ng of recombinant SEA (rSEA) protein. Group (3) consisted of mice injected

intramuscularly with 100 μg of PCR_3-SEA.S DNA (50 μg per leg, total of 100 μg/mouse). Group (4) consisted of mice injected intramuscularly with 100 μg PCR_3 (no insert; mock) DNA (50 μg per leg, total of 100 μg/mouse). The DNA samples were prepared by diluting 100 μl of a solution containing 100 μg of DNA 50:50 (v:v) in sterile PBS prior to injection. The rSEA protein was purified from cultures of *E. coli* cells transformed with the recombinant molecule PKK223 (obtained from Dr. John Kappler) encoding the truncated SEA.S protein lacking a leader sequence.

Beginning 72 hours after injection, mice were tail bled and PBMC prepared for fluorescence activated cell sorter (FACS) analysis. Cells were double labeled with the monoclonal antibodies FITC conjugated-GK1.5 antibody, biotinylated-KJ25 antibody and biotinylated-F23.1, to analyze for expression of CD4, TCR Vβ3 and TCR Vβ8 expression, respectively. The labelled cells were analyzed on an EPICS-C flow cytometer.

The percentage of cells isolated from the experimental mice expressing CD4 that also expressed either Vβ8 or Vβ3 was calculated and compared to percentages expressed by cells isolated from control mice. The mean percentage of CD4+ and Vβ3+ T cells in PBMC was plotted against time after injection. The results are shown in Fig. 8 and indicate that the percentage of CD4+, Vβ3+ T cells declined rapidly in PBMC of mice that received intramuscular injections with PCR_3-SEA.S DNA, but not in mice mock

injected with mock DNA. The percentages of Vβ8+ cells was not affected. This result is predicted since SEA protein does not bind mouse Vβ8+ T cells. The decline of the percentage of CD4+, Vβ3+ T cells occurred as rapidly as in mice injected with the recombinant SEA protein (rSEA). The deletion, however, observed over the next 2 months in mice injected with PCR_3-SEA.S DNA was longer lasting and was more pronounced than the deletion induced by injection of SEA.S protein. In addition, injection of as little as 2 μg PCR_3-SEA.S DNA also induced deletion of Vβ3+ T cells. Thus, intramuscular injection of DNA encoding superantigens represents a potent and non-toxic approach to the deletion or suppression of potentially harmful (e.g., autoreactive T cells) T cells.

Example 12

This example describes the production of immunogen and chemokine encoding recombinant molecules.

Recombinant molecules encoding ovalbumin (OVA) were produced by ligating cDNA encoding OVA into the eukaryotic expression vector PCR_3 and is referred to herein as PCR_3-OVA. cDNA encoding murine RANTES, murine macrophage inflammatory protein-1 alpha (MIP-1α), and macrophage inflammatory protein-1 beta (MIP-1β) was prepared from RNA isolated from LPS-stimulated normal murine bone marrow macrophages using methods standard in the art. The cDNA were ligated into the expression vector PCR_3, and are referred to herein as PCR_3-RANTES, PCR_3-MIP-1α and PCR_3-MIP-

1β. All plasmid DNA were purified by cesium chloride gradient centrifugation and resuspended at 1.0 mg/ml in sterile PBS.

Example 13

This example demonstrates that the co-administration of adjuvant DNA and immunogen DNA stimulates antibody production against the immunogen protein.

Separate groups of 4 CB6 F1 mice per group were injected twice with the following mixtures of DNA: (1) about 100 μg PCR_3-OVA + about 100 μg PCR_3-MIP-1β; (2) about 100 μg PCR_3-OVA + about 50 μg PCR_3-SEB (described in Example 1) + PCR_3-GM-CSF (described in Example 1); (3) about 100 μg PCR_3-OVA + about 100 μg PCR_3-RANTES; (4) about 100 μg PCR_3-OVA + about 100 μg PCR_3-SEB; (5) about 100 μg PCR_3-OVA + about 100 μg PCR_3-GM-CSF; or (6) about 100 μg PCR_3-OVA alone. Control samples were also prepared which included 6 non-injected, syngeneic mice. The DNA was diluted to a final concentration of 0.5 mg/ml in sterile phosphate buffered saline (PBS) prior to injection. The mice were injected intramuscularly, bilaterally in their quadriceps muscles (about 100 μg of DNA per quadricep).

About 20 days after the immunization of step B, serum was collected from each mouse and assayed for antibodies that specifically bind to OVA protein using an OVA-specific enzyme linked immunoassay (ELISA) assay using methods standard in the art. Briefly, OVA protein was bound to an ELISA plate. The plates were washed and then incubated in

the presence of serum. Again the plates were washed and then incubated in the presence of HRP-conjugated anti-mouse IgG antibody. The amount of antibody bound to the OVA was detected on an ELISA reader and are expressed in absorbance units.

The results of the ELISA are shown in Fig. 9 and indicate that co-injection of DNA encoding OVA, with either DNA encoding RANTES or MIP-1β, or SEB and GM-CSF, increases the antibody response to OVA over that observed with OVA alone, OVA plus GM-CSF, OVA plus SEB alone or control samples. Thus, the expression of RANTES, MIP-1β, or SEB and GM-CSF increase the antibody response to OVA when administered as a DNA vaccine.

Example 14

This example demonstrates that the co-administration of DNA adjuvant and immunogen DNA results in the production of interferon gamma.

Separate groups of 4 CB6 F1 mice per group were injected twice, intramuscularly (on day 1 and day 21), with the following mixtures of DNA: (1) about 100 μg PCR_3-OVA + about 100 μg PCR_3-MIP-1β; (2) about 100 μg PCR_3-OVA + about 50 μg PCR_3-SEB + PCR_3-GM-CSF; (3) about 100 μg PCR_3-OVA + about 100 μg PCR_3-RANTES; (4) about 100 μg PCR_3-OVA + about 100 μg PCR_3-SEB; (5) about 100 μg PCR_3-OVA + about 100 μg PCR_3-GM-CSF; or (6) about 100 μg PCR_3-OVA alone. Control samples were also prepared as above.

The mice were sacrificed on day 27. Spleen cells were harvested from each mouse and re-stimulated *in vitro* with irradiated OVA-transfected cells (EG7-OVA) in quadruplicate wells. On day 4 of the re-stimulation with irradiated EG7-OVA cells, supernatants were harvested from the cultures and assayed for interferon gamma activity using an interferon gamma-specific ELISA assay. Results were expressed as units/ml of interferon activity, as determined by comparison with a standard curve generated with recombinant murine interferon-gamma.

The results are shown in Fig. 10 and indicate that RANTES or GM-CSF were effective compounds for inducing interferon-gamma production. Although less, SEB and MIP-1β also induced interferon-gamma production. Additional experiments indicated that none of the adjuvants evaluated in this experiment induced significant quantities of IL-4 release. Together, these data indicate that the immune response induced by an adjuvant of the present invention is primarily a Th1 response, which induces primarily cell-mediated immunity, including macrophage activation, enhanced T cell CTL activity, and increased MHC expression.

Example 15

This example demonstrates that the co-administration of adjuvant DNA and immunogen DNA induce T cell proliferative responses to the immunogen.

Separate group of 4 CB6 F1 mice per group were immunized using the protocol described in Example 14. The

animals were sacrificed on day 27 and harvested spleen cells re-stimulated using the method described in Example 14. After about 4 days of re-stimulation, 100 μl aliquots of the cells were harvested from each well and pulsed for 18 hours with ^{3}H-thymidine. Thymidine incorporation was then quantitated (cpm) as a measure of the proliferative response to OVA expressed by the transfected EG7-OVA cell line.

The results are shown in Fig. 11 and indicate that MIP-1β, RANTES, SEB + GM-CSF, and SEB alone, when co-administered together with OVA DNA, induce a substantial increase in the proliferative response to OVA. Thus, these data provide evidence that DNA encoding chemokines and SAgs are useful for enhancing cell-mediated immune responses and therefore are useful as DNA vaccine adjuvants.

Example 16

This example demonstrates that the co-administration of adjuvant DNA increases CTL responses to the immunogen ovalbumin.

Mice were immunized using the protocol described in Example 14. Spleen cells were harvested from the immunized mice 7 days after the last vaccination. The cells were then re-stimulated *in vitro* for 6 days with irradiated EG7-OVA cells. T cells were then harvested from the re-stimulated population and added in decreasing numbers to ^{51}Cr-labeled EG7-OVA or EL-4 target cells in a standard 4 hour chromium release assay for CTL activity. The percent

cell lysis was determined Chromium release was then quantitated (cpm) as a measure of the percent specific cell lysis of labeled target cells. The higher the % specific lysis, the more CTL activity exhibited by the T cells.

The results are shown in Fig. 12 and indicate that all of the adjuvant DNAs evaluated induced increased CTL activity compared to OVA alone. The use of RANTES, GM-CSF and SEB alone, each were effective in inducing CTL activity. These data indicate that co-administration of chemokine DNA can enhance CTL-mediated immunity to an intracellular immunogen, as typified by OVA expressed in a transfected cell line, indicating that this approach is useful for vaccines against intracellular pathogens.

Taken together, the results of Examples 12-16 indicate that all DNA adjuvants tested (GM-CSF, SEB, SEB+GM-CSF, RANTES and MIP-1β) improved cell mediated immunity against the immunogen ovalbumin. In particular, the use of either SEB or GM-CSF alone, as well as the combination of SEB + GM-CSF were effective at inducing cell mediated immunity.

SEQUENCE LISTING

The following Sequence Listing is submitted pursuant to 37 CFR §1.821. A copy in computer readable form is also submitted herewith.

Applicants assert pursuant to 37 CFR §1.821(f) that the content of the paper and computer readable copies of SEQ ID NO:1 through SEQ ID NO:13 submitted herewith are the same.

(1) GENERAL INFORMATION:

(i) APPLICANT: Dow, Steve W.
Elmslie, Robyn E.
Potter, Terence A.

(ii) TITLE OF INVENTION: GENE THERAPY FOR EFFECTOR CELL REGULATION

(iii) NUMBER OF SEQUENCES: 13

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Sheridan Ross & McIntosh
(B) STREET: 1700 Lincoln Street, Suite 3500
(C) CITY: Denver
(D) STATE: Colorado
(E) COUNTRY: U.S.A.
(F) ZIP: 80203

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Connell, Gary J.
(B) REGISTRATION NUMBER: 32,020
(C) REFERENCE/DOCKET NUMBER: 2879-29-C1-PCT

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (303) 863-9700
(B) TELEFAX: (303) 863-0223

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 773 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..765

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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ATG ACC ATG ATT ACG AAT TTA ATA CGA CTC ACT ATA GGG AAT TCC ATG      48
Met Thr Met Ile Thr Asn Leu Ile Arg Leu Thr Ile Gly Asn Ser Met
 1               5                  10                  15

GAG AGT CAA CCA GAT CCT AAA CCA GAT GAG TTG CAC AAA TCG AGT AAA      96
Glu Ser Gln Pro Asp Pro Lys Pro Asp Glu Leu His Lys Ser Ser Lys
            20                  25                  30

TTC ACT GGT TTG ATG GAA AAT ATG AAA GTT TTG TAT GAT GAT AAT CAT     144
Phe Thr Gly Leu Met Glu Asn Met Lys Val Leu Tyr Asp Asp Asn His
        35                  40                  45

GTA TCA GCA ATA AAC GTT AAA TCT ATA GAT CAA TTT CTA TAC TTT GAC     192
Val Ser Ala Ile Asn Val Lys Ser Ile Asp Gln Phe Leu Tyr Phe Asp
    50                  55                  60

TTA ATA TAT TCT ATT AAG GAC ACT AAG TTA GGG AAT TAT GAT AAT GTT     240
Leu Ile Tyr Ser Ile Lys Asp Thr Lys Leu Gly Asn Tyr Asp Asn Val
65                  70                  75                  80

CGA GTC GAA TTT AAA AAC AAA GAT TTA GCT GAT AAA TAC AAA GAT AAA     288
Arg Val Glu Phe Lys Asn Lys Asp Leu Ala Asp Lys Tyr Lys Asp Lys
                85                  90                  95

TAC GTA GAT GTG TTT GGA GCT AAT TAT TAT TAT CAA TGT TAT TTT TCT     336
Tyr Val Asp Val Phe Gly Ala Asn Tyr Tyr Tyr Gln Cys Tyr Phe Ser
            100                 105                 110

AAA AAA ACG AAT GAT ATT AAT TCG CAT CAA ACT GAC AAA CGA AAA ACT     384
Lys Lys Thr Asn Asp Ile Asn Ser His Gln Thr Asp Lys Arg Lys Thr
        115                 120                 125

TGT ATG TAT GGT GGT GTA ACT GAG CAT AAT GGA AAC CAA TTA GAT AAA     432
Cys Met Tyr Gly Gly Val Thr Glu His Asn Gly Asn Gln Leu Asp Lys
    130                 135                 140

TAT AGA AGT ATT ACT GTT CGG GTA TTT GAA GAT GGT AAA AAT TTA TTA     480
Tyr Arg Ser Ile Thr Val Arg Val Phe Glu Asp Gly Lys Asn Leu Leu
145                 150                 155                 160

TCT TTT GAC GTA CAA ACT AAT AAG AAA AAG GTG ACT GCT CAA GAA TTA     528
Ser Phe Asp Val Gln Thr Asn Lys Lys Lys Val Thr Ala Gln Glu Leu
                165                 170                 175

GAT TAC CTA ACT CGT CAC TAT TTG GTG AAA AAT AAA AAA CTC TAT GAA     576
Asp Tyr Leu Thr Arg His Tyr Leu Val Lys Asn Lys Lys Leu Tyr Glu
            180                 185                 190
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TTT AAC AAC TCG CCT TAT GAA ACG GGA TAT ATT AAA TTT ATA GAA AAT 624
Phe Asn Asn Ser Pro Tyr Glu Thr Gly Tyr Ile Lys Phe Ile Glu Asn
195 200 205

GAG AAT AGC TTT TGG TAT GAC ATG ATG CCT GCA CCA GGA GAT AAA TTT 672
Glu Asn Ser Phe Trp Tyr Asp Met Met Pro Ala Pro Gly Asp Lys Phe
210 215 220

GAC CAA TCT AAA TAT TTA ATG ATG TAC AAT GAC AAT AAA ATG GTT GAT 720
Asp Gln Ser Lys Tyr Leu Met Met Tyr Asn Asp Asn Lys Met Val Asp
225 230 235 240

TCT AAA GAT GTG AAG ATT GAA GTT TAT CTT ACG ACA AAG AAA AAG 765
Ser Lys Asp Val Lys Ile Glu Val Tyr Leu Thr Thr Lys Lys Lys
245 250 255

TGAAGCTT 773

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 255 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Thr Met Ile Thr Asn Leu Ile Arg Leu Thr Ile Gly Asn Ser Met
1 5 10 15

Glu Ser Gln Pro Asp Pro Lys Pro Asp Glu Leu His Lys Ser Ser Lys
20 25 30

Phe Thr Gly Leu Met Glu Asn Met Lys Val Leu Tyr Asp Asp Asn His
35 40 45

Val Ser Ala Ile Asn Val Lys Ser Ile Asp Gln Phe Leu Tyr Phe Asp
50 55 60

Leu Ile Tyr Ser Ile Lys Asp Thr Lys Leu Gly Asn Tyr Asp Asn Val
65 70 75 80

Arg Val Glu Phe Lys Asn Lys Asp Leu Ala Asp Lys Tyr Lys Asp Lys
85 90 95

Tyr Val Asp Val Phe Gly Ala Asn Tyr Tyr Tyr Gln Cys Tyr Phe Ser
100 105 110

Lys Lys Thr Asn Asp Ile Asn Ser His Gln Thr Asp Lys Arg Lys Thr
115 120 125

Cys Met Tyr Gly Gly Val Thr Glu His Asn Gly Asn Gln Leu Asp Lys
130 135 140

Tyr Arg Ser Ile Thr Val Arg Val Phe Glu Asp Gly Lys Asn Leu Leu
145 150 155 160

Ser Phe Asp Val Gln Thr Asn Lys Lys Lys Val Thr Ala Gln Glu Leu
165 170 175

Asp Tyr Leu Thr Arg His Tyr Leu Val Lys Asn Lys Lys Leu Tyr Glu
180 185 190

```
Phe Asn Asn Ser Pro Tyr Glu Thr Gly Tyr Ile Lys Phe Ile Glu Asn
        195                 200                 205

Glu Asn Ser Phe Trp Tyr Asp Met Met Pro Ala Pro Gly Asp Lys Phe
    210                 215                 220

Asp Gln Ser Lys Tyr Leu Met Met Tyr Asn Asp Asn Lys Met Val Asp
225                 230                 235                 240

Ser Lys Asp Val Lys Ile Glu Val Tyr Leu Thr Thr Lys Lys Lys
                245                 250                 255
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 751 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 46..744

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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ATGACCATGA TTACGAATTT AATACGACTC ACTATAGGGA ATTCC ATG GAG AAA      54
                                                  Met Glu Lys
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AGC GAA GAA ATA AAT GAG AAA GAT CTG CGC AAG AAG TCC GAA TTG CAG    102
Ser Glu Glu Ile Asn Glu Lys Asp Leu Arg Lys Lys Ser Glu Leu Gln
  5                  10                  15

GGA ACA GCC CTA GGC AAT CTT AAA CAA ATC TAT TAT TAC AAT GAA AAA    150
Gly Thr Ala Leu Gly Asn Leu Lys Gln Ile Tyr Tyr Tyr Asn Glu Lys
 20                  25                  30                  35

GCG AAG ACT GAG AAT AAA GAG AGT CAC GAT CAA TTT CTG CAG CAT ACT    198
Ala Lys Thr Glu Asn Lys Glu Ser His Asp Gln Phe Leu Gln His Thr
                40                  45                  50

ATA TTG TTT AAA GGC TTT TTT ACT GAT CAT TCG TGG TAT AAC GAT TTA    246
Ile Leu Phe Lys Gly Phe Phe Thr Asp His Ser Trp Tyr Asn Asp Leu
            55                  60                  65

CTA GTA GAT TTT GAT TCG AAG GAC ATC GTT GAT AAA TAT AAA GGG AAG    294
Leu Val Asp Phe Asp Ser Lys Asp Ile Val Asp Lys Tyr Lys Gly Lys
        70                  75                  80

AAG GTC GAC TTG TAT GGT GCT TAT TAT GGG TAC CAA TGT GCT GGT GGT    342
Lys Val Asp Leu Tyr Gly Ala Tyr Tyr Gly Tyr Gln Cys Ala Gly Gly
  85                  90                  95

ACA CCA AAC AAA ACA GCA TGC ATG TAT GGT GGG GTA ACC TTA CAT GAC    390
Thr Pro Asn Lys Thr Ala Cys Met Tyr Gly Gly Val Thr Leu His Asp
100                 105                 110                 115

AAT AAT CGA TTG ACC GAA GAG AAA AAG GTC CCG ATC AAT TTA TGG CTA    438
Asn Asn Arg Leu Thr Glu Glu Lys Lys Val Pro Il  Asn Leu Trp Leu
                120                 125                 130
```

```
GAC GGT AAA CAA AAT ACA GTA CCT CTA GAA ACG GTT AAA ACG AAT AAG      486
Asp Gly Lys Gln Asn Thr Val Pro Leu Glu Thr Val Lys Thr Asn Lys
          135                 140                 145

AAA AAT GTA ACT GTC CAA GAG CTG GAT CTT CAA GCG CGC CGA TAC CTA      534
Lys Asn Val Thr Val Gln Glu Leu Asp Leu Gln Ala Arg Arg Tyr Leu
      150                 155                 160

CAG GAA AAA TAT AAT TTG TAC AAC TCT GAC GTC TTT GAT GGG AAG GTT      582
Gln Glu Lys Tyr Asn Leu Tyr Asn Ser Asp Val Phe Asp Gly Lys Val
  165                 170                 175

CAG AGA GGC CTA ATC GTG TTT CAT ACT TCT ACA GAA CCT TCG GTT AAC      630
Gln Arg Gly Leu Ile Val Phe His Thr Ser Thr Glu Pro Ser Val Asn
180                 185                 190                 195

TAC GAT TTA TTT GGA GCT CAA GGA CAG TAT TCA AAT ACA CTC TTA AGA      678
Tyr Asp Leu Phe Gly Ala Gln Gly Gln Tyr Ser Asn Thr Leu Leu Arg
                200                 205                 210

ATA TAT CGC GAC AAC AAG ACG ATT AAC TCT GAA AAC ATG CAC ATT GAT      726
Ile Tyr Arg Asp Asn Lys Thr Ile Asn Ser Glu Asn Met His Ile Asp
          215                 220                 225

ATC TAT TTA TAT ACA AGT TAAGCTT                                     751
Ile Tyr Leu Tyr Thr Ser
      230
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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 233 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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Met Glu Lys Ser Glu Glu Ile Asn Glu Lys Asp Leu Arg Lys Lys Ser
 1               5                  10                  15

Glu Leu Gln Gly Thr Ala Leu Gly Asn Leu Lys Gln Ile Tyr Tyr Tyr
            20                  25                  30

Asn Glu Lys Ala Lys Thr Glu Asn Lys Glu Ser His Asp Gln Phe Leu
        35                  40                  45

Gln His Thr Ile Leu Phe Lys Gly Phe Phe Thr Asp His Ser Trp Tyr
    50                  55                  60

Asn Asp Leu Leu Val Asp Phe Asp Ser Lys Asp Ile Val Asp Lys Tyr
65                  70                  75                  80

Lys Gly Lys Lys Val Asp Leu Tyr Gly Ala Tyr Tyr Gly Tyr Gln Cys
                85                  90                  95

Ala Gly Gly Thr Pro Asn Lys Thr Ala Cys Met Tyr Gly Gly Val Thr
            100                 105                 110

Leu His Asp Asn Asn Arg Leu Thr Glu Glu Lys Lys Val Pro Ile Asn
        115                 120                 125

Leu Trp Leu Asp Gly Lys Gln Asn Thr Val Pro Leu Glu Thr Val Lys
    130                 135                 140
```

```
Thr Asn Lys Lys Asn Val Thr Val Gln Glu Leu Asp Leu Gln Ala Arg
145                 150                 155                 160

Arg Tyr Leu Gln Glu Lys Tyr Asn Leu Tyr Asn Ser Asp Val Phe Asp
                165                 170                 175

Gly Lys Val Gln Arg Gly Leu Ile Val Phe His Thr Ser Thr Glu Pro
            180                 185                 190

Ser Val Asn Tyr Asp Leu Phe Gly Ala Gln Gly Gln Tyr Ser Asn Thr
        195                 200                 205

Leu Leu Arg Ile Tyr Arg Asp Asn Lys Thr Ile Asn Ser Glu Asn Met
    210                 215                 220

His Ile Asp Ile Tyr Leu Tyr Thr Ser
225                 230
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 582 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..582

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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 1               5                  10                  15

TCT GAC ACT TTT ACA AAT AGT GAA GTT TTA GAT AAT TCC TTA GGA TCT      96
Ser Asp Thr Phe Thr Asn Ser Glu Val Leu Asp Asn Ser Leu Gly Ser
            20                  25                  30

ATG CGT ATA AAA AAC ACA GAT GGC AGC ATC AGC CTT ATA ATT TTT CCG     144
Met Arg Ile Lys Asn Thr Asp Gly Ser Ile Ser Leu Ile Ile Phe Pro
        35                  40                  45

AGT CCT TAT TAT AGC CCT GCT TTT ACA AAA GGG GAA AAA GTT GAC TTA     192
Ser Pro Tyr Tyr Ser Pro Ala Phe Thr Lys Gly Glu Lys Val Asp Leu
    50                  55                  60

AAC ACA AAA AGA ACT AAA AAA AGC CAA CAT ACT AGC GAA GGA ACT TAT     240
Asn Thr Lys Arg Thr Lys Lys Ser Gln His Thr Ser Glu Gly Thr Tyr
65                  70                  75                  80

ATC CAT TTC CAA ATA AGT GGC GTT ACA AAT ACT GAA AAA TTA CCT ACT     288
Ile His Phe Gln Ile Ser Gly Val Thr Asn Thr Glu Lys Leu Pro Thr
                85                  90                  95

CCA ATA GAA CTA CCT TTA AAA GTT AAG GTT CAT GGT AAA GAT AGC CCC     336
Pro Ile Glu Leu Pro Leu Lys Val Lys Val His Gly Lys Asp Ser Pro
            100                 105                 110

TTA AAG TAT TGG CCA AAG TTC GAT AAA AAA CAA TTA GCT ATA TCA ACT     384
Leu Lys Tyr Trp Pro Lys Phe Asp Lys Lys Gln Leu Ala Ile Ser Thr
        115                 120                 125
```

```
TTA GAC TTT GAA ATT CGT CAT CAG CTA ACT CAA ATA CAT GGA TTA TAT      432
Leu Asp Phe Glu Ile Arg His Gln Leu Thr Gln Ile His Gly Leu Tyr
    130                 135                 140

CGT TCA AGC GAT AAA ACG GGT GGT TAT TGG AAA ATA ACA ATG AAT GAC      480
Arg Ser Ser Asp Lys Thr Gly Gly Tyr Trp Lys Ile Thr Met Asn Asp
145                 150                 155                 160

GGA TCC ACA TAT CAA AGT GAT TTA TCT AAA AAG TTT GAA TAC AAT ACT      528
Gly Ser Thr Tyr Gln Ser Asp Leu Ser Lys Lys Phe Glu Tyr Asn Thr
                165                 170                 175

GAA AAA CCA CCT ATA AAT ATT GAT GAA ATA AAA ACT ATA GAA GCA GAA      576
Glu Lys Pro Pro Ile Asn Ile Asp Glu Ile Lys Thr Ile Glu Ala Glu
            180                 185                 190

ATT AAT                                                             582
Ile Asn
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(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 194 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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Met Thr Asn Asp Asn Ile Lys Asp Leu Leu Asp Trp Tyr Ser Ser Gly
 1               5                  10                  15

Ser Asp Thr Phe Thr Asn Ser Glu Val Leu Asp Asn Ser Leu Gly Ser
            20                  25                  30

Met Arg Ile Lys Asn Thr Asp Gly Ser Ile Ser Leu Ile Ile Phe Pro
        35                  40                  45

Ser Pro Tyr Tyr Ser Pro Ala Phe Thr Lys Gly Glu Lys Val Asp Leu
    50                  55                  60

Asn Thr Lys Arg Thr Lys Lys Ser Gln His Thr Ser Glu Gly Thr Tyr
65                  70                  75                  80

Ile His Phe Gln Ile Ser Gly Val Thr Asn Thr Glu Lys Leu Pro Thr
                85                  90                  95

Pro Ile Glu Leu Pro Leu Lys Val Lys Val His Gly Lys Asp Ser Pro
            100                 105                 110

Leu Lys Tyr Trp Pro Lys Phe Asp Lys Lys Gln Leu Ala Ile Ser Thr
        115                 120                 125

Leu Asp Phe Glu Ile Arg His Gln Leu Thr Gln Ile His Gly Leu Tyr
    130                 135                 140

Arg Ser Ser Asp Lys Thr Gly Gly Tyr Trp Lys Ile Thr Met Asn Asp
145                 150                 155                 160

Gly Ser Thr Tyr Gln Ser Asp Leu Ser Lys Lys Phe Glu Tyr Asn Thr
                165                 170                 175

Glu Lys Pro Pro Ile Asn Ile Asp Glu Ile Lys Thr Ile Glu Ala Glu
            180                 185                 190

Ile Asn
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..25
(D) OTHER INFORMATION: /label= primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GGGAATTCCA TGGAGAGTCA ACCAG 25

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..23
(D) OTHER INFORMATION: /label= primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GCGGATCCTC ACTTTTTCTT TGT 23

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..22
(D) OTHER INFORMATION: /label= primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GGGAATTCCA TGGAGAAAAG CG 22

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..25
(D) OTHER INFORMATION: /label= primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GCAAGCTTAA CTTGTATATA AATAG 25

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..51

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CGGGGTACCC CGAAGGAGGA AAAAAAAATG TCTACAAACG ATAATATAAA G 51

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..42
(D) OTHER INFORMATION: /label= primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TGCTCTAGAG CATTAATTAA TTTCTGCTTC TATAGTTTTT AT 42

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 279 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ACCATGAAGA TCTCTGCAGC TGCCCTCACC ATCATCCTCA CTGCAGCCGC CCTCTGGGCG 60

CCCGCGCCTG CCTCACCATA TGGCTCGGAC ACCACTCCCT GCTGCTTTGC CTACCTCTCC 120

CTCGCGCTGC CTCGTGCCCA CGTCAAGGAG TATTTCTACA CCAGCAGCAA GTGCTCCAAT 180

CTTGCAGTCG TGTTTGTCAC TCGAAGGAAC CGCCAAGTGT GTGCCAACCC AGAGAAGAAG 240

TGGGTTCAAG AATACATCAA CTATTTGGAG ATGAGCTAG 279

While various embodiments of the present invention have been described in detail, it is apparent that modifications and adaptations of those embodiments will occur to those skilled in the art. It is to be expressly understood, however, that such modifications and adaptations are within the scope of the present invention, as set forth in the following claims:

What is claimed:

1. A therapeutic composition comprising: (a) an isolated nucleic acid molecule encoding a superantigen; and (b) an isolated nucleic acid molecule selected from the group consisting of a nucleic acid molecule encoding a cytokine, a nucleic acid molecule encoding a chemokine and mixtures thereof, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences.

2. The therapeutic composition of Claim 1, wherein said superantigen is selected from the group consisting of staphylococcal enterotoxins, retroviral antigens, streptococcal antigens, mycoplasma antigens, mycobacteria antigens, viral antigens and protozoan antigens.

3. The therapeutic composition of Claim 1, wherein said superantigen comprises staphylococcal enterotoxins.

4. The therapeutic composition of Claim 1, wherein said superantigen is selected from the group consisting of SEA, SEB, SEC_1, SEC_2, SEC_3, SED, SEE and TSST.

5. The therapeutic composition of Claim 1, wherein said superantigen is derived from a virus selected from the group consisting of mouse mammary tumor virus, rabies virus and herpes virus.

6. The therapeutic composition of Claim 1, wherein said cytokine is selected from the group consisting of hematopoietic growth factors, interleukins, interferons,

immunoglobulin superfamily molecules, tumor necrosis factor family molecules and chemokines.

7. The therapeutic composition of Claim 1, wherein said cytokine is selected from the group consisting of granulocyte macrophage colony stimulating factor, macrophage colony stimulating factor, tumor necrosis factor α, interleukin-1, interleukin-2, interleukin-4, interleukin-6, interleukin-12, and interleukin-15.

8. The therapeutic composition of Claim 1, wherein said cytokine is selected from the group consisting of granulocyte macrophage colony stimulating factor and tumor necrosis factor α.

9. The therapeutic composition of Claim 1, wherein said cytokine comprises granulocyte macrophage colony stimulating factor.

10. The therapeutic composition of Claim 1, wherein said chemokine is selected from the group consisting of C5a, IL-8, MIP1α, MIP1β, MCP-1, MCP-3, PAFR, FMLPR, LTB_4R, GRP, RANTES, eotaxin, lymphotactin, IP10, I-309, ENA78, GCP-2, NAP-2 and MGSA/gro.

11. The therapeutic composition of Claim 1, wherein said chemokine is selected from the group consisting of IL-8, MIP1α, MIP1β, MCP-1, MCP-3, RANTES and NAP-2.

12. The therapeutic composition of Claim 1, wherein said chemokine is selected from the group consisting of IL-8, Rantes, MIP1α and MIP1β.

13. The therapeutic composition of Claim 1, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences capable of being expressed in a mammalian cell.

14. The therapeutic composition of Claim 1, wherein said transcription control sequences are selected from the group consisting of RSV control sequences, CMV control sequences, retroviral LTR sequences, SV-40 control sequences and β-actin control sequences.

15. The therapeutic composition of Claim 1, wherein said isolated nucleic acid molecule encoding a superantigen comprises a recombinant molecule selected from the group consisting of PCR_3-SEB, PCR_3-SEA, PCR_3-SEB.S, PCR_3-SEA.S and PCR_3-TSST.

16. The therapeutic composition of Claim 1, wherein said isolated nucleic acid molecule encoding a cytokine comprises PCR_3-GM_3.

17. The therapeutic composition of Claim 1, wherein said isolated nucleic acid molecule encoding a chemokine is selected from the group consisting of PCR_3-RANTES, PCR_3-MIP1α and PCR_3-MIP1β.

18. The therapeutic composition of Claim 1, wherein said therapeutic composition further comprises a pharmaceutically acceptable carrier selected from the group consisting of an aqueous physiologically balanced solution, an artificial lipid-containing substrate, a natural lipid-

containing substrate, an oil, an ester, a glycol, a virus and a metal particle.

19. The therapeutic composition of Claim 18, wherein said pharmaceutically acceptable carrier is selected from the group consisting of liposomes and an aqueous physiologically balanced solution.

20. The therapeutic composition of Claim 1, wherein said therapeutic composition further comprises an immunogen selected from the group consisting of a pathogen, an allergen, tumor antigens and self-antigens.

21. The therapeutic composition of Claim 1, wherein said therapeutic composition further comprises an immunogen comprising a peptide derived from *Mycobacterium tuberculosis*.

22. A recombinant molecule comprising: (a) a first isolated nucleic acid molecule encoding a superantigen; and (b) a second isolated nucleic acid molecule selected from the group consisting of a nucleic acid molecule encoding a cytokine, a nucleic acid molecule encoding a chemokine, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences.

23. The molecule of Claim 22, wherein said recombinant molecule contains a transcription control sequence selected from the group consisting of RSV control sequences, CMV control sequences, retroviral LTR control

sequences, SV-40 control sequences and β-actin control sequences.

24. The molecule of Claim 22, wherein said recombinant molecule is dicistronic and comprises an IRES.

25. The molecule of Claim 22, wherein said first nucleic acid molecule and said second nucleic acid molecule are separated by an IRES.

26. The molecule of Claim 22, wherein said first nucleic acid molecule encodes a superantigen selected from the group consisting of SEA, SEB, SEC_1, SEC_2, SEC_3, SED, SEE and TSST.

27. The molecule of Claim 22, wherein said second nucleic acid molecule encodes a protein selected from the group consisting of macrophage colony stimulating factor, macrophage colony stimulating factor, tumor necrosis factor α, interleukin-1, interleukin-2, interleukin-4, interleukin-6, interleukin-12, interleukin-15, C5a, IL-8, MIP1α, MIP1β, MCP-1, MCP-3, PAFR, FMLPR, LTB_4R, GRP, RANTES, eotaxin, lymphotactin, IP10, I-309, ENA78, GCP-2, NAP-2 and MGSA/gro.

28. A recombinant molecule comprising: (a) a first isolated nucleic acid molecule encoding a first superantigen; and (b) a second isolated nucleic acid molecule encoding a second superantigen, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences.

29. The molecule of Claim 28, wherein said recombinant molecule contains a transcription control sequence selected from the group consisting of RSV control sequences, CMV control sequences, retroviral LTR control sequences, SV-40 control sequences and β-actin control sequences.

30. The molecule of Claim 28, wherein said recombinant molecule is dicistronic and comprises an IRES.

31. The molecule of Claim 28, wherein said first nucleic acid molecule and said second nucleic acid molecule are separated by an IRES.

32. The molecule of Claim 28, wherein said first nucleic acid molecule encodes a superantigen selected from the group consisting of SEA, SEB, SEC_1, SEC_2, SEC_3, SED, SEE and TSST.

33. A therapeutic composition comprising a delivery vehicle carrying: (a) a first isolated nucleic acid molecule encoding a superantigen; and (b) a second isolated nucleic acid molecule selected from the group consisting of a nucleic acid molecule encoding a cytokine, a nucleic acid molecule encoding a chemokine and mixtures thereof, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences.

34. The therapeutic composition of Claim 33, wherein said delivery vehicle comprises a liposome.

35. The therapeutic composition of Claim 33, wherein said first nucleic acid molecule encodes a superantigen

selected from the group consisting of SEA, SEB, SEC_1, SEC_2, SEC_3, SED, SEE and TSST.

36. The therapeutic composition of Claim 33, wherein said second nucleic acid molecule encodes a protein selected from the group consisting of macrophage colony stimulating factor, macrophage colony stimulating factor, tumor necrosis factor α, interleukin-1, interleukin-2, interleukin-4, interleukin-6, interleukin-12, interleukin-15, C5a, IL-8, MIP1α, MIP1β, MCP-1, MCP-3, PAFR, FMLPR, LTB_4R, GRP, RANTES, eotaxin, lymphotactin, IP10, I-309, ENA78, GCP-2, NAP-2 and MGSA/gro.

37. The therapeutic composition of Claim 33, wherein said delivery vehicle further carries a nucleic acid molecule encoding an immunogen.

38. The therapeutic composition of Claim 33, wherein said delivery vehicle further carries a peptide derived from *Mycobacterium tuberculosis*.

39. A therapeutic composition comprising a delivery vehicle carrying an isolated nucleic acid molecule encoding a superantigen, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences.

40. The therapeutic composition of Claim 39, wherein said delivery vehicle comprises a liposome.

41. The therapeutic composition of Claim 39, wherein said nucleic acid molecule encodes a superantigen selected

from the group consisting of SEA, SEB, SEC_1, SEC_2, SEC_3, SED, SEE and TSST.

42. The therapeutic composition of Claim 39, wherein said delivery vehicle further carries a nucleic acid molecule encoding a protein selected from the group consisting of a cytokine, a chemokine and an immunogen.

43. The therapeutic composition of Claim 39, wherein said delivery vehicle further carries a nucleic acid molecule encoding a protein selected from the group consisting of macrophage colony stimulating factor, macrophage colony stimulating factor, tumor necrosis factor α, interleukin-1, interleukin-2, interleukin-4, interleukin-6, interleukin-12, interleukin-15, C5a, IL-8, MIP1α, MIP1β, MCP-1, MCP-3, PAFR, FMLPR, LTB_4R, GRP, RANTES, eotaxin, lymphotactin, IP10, I-309, ENA78, GCP-2, NAP-2 and MGSA/gro.

44. The therapeutic composition of Claim 39, wherein said delivery vehicle further carries a peptide derived from *Mycobacterium tuberculosis*.

45. An adjuvant composition, comprising an immunogen and an isolated nucleic acid molecule encoding a superantigen, wherein said isolated nucleic acid molecule is operatively linked to one or more transcription control sequences.

46. The adjuvant composition of Claim 45, wherein said adjuvant composition further comprises a nucleic acid

molecule encoding a protein selected from the group consisting of a cytokine, a chemokine and mixtures thereof.

47. The adjuvant composition of Claim 45, wherein said immunogen comprises a compound selected from the group consisting of a nucleic acid molecule and a peptide.

48. The adjuvant composition of Claim 45, wherein said adjuvant composition comprises up to about 50% of a nucleic acid molecule encoding an immunogen and up to about 50% of a nucleic acid molecule encoding a superantigen.

49. The adjuvant composition of Claim 45, wherein said adjuvant composition comprises up to about 66% of a nucleic acid molecule encoding an immunogen and up to about 33% of a nucleic acid molecule encoding a superantigen.

50. The adjuvant composition of Claim 45, wherein said adjuvant composition comprises up to about 50% of a nucleic acid molecule encoding an immunogen, up to about 25% of a nucleic acid molecule encoding a superantigen and up to about 25% of a nucleic acid molecule selected from the group consisting of a nucleic acid molecule encoding a cytokine, a nucleic acid molecule encoding a chemokine and mixtures thereof.

51. The adjuvant composition of Claim 45, wherein said nucleic acid molecules comprise naked DNA.

52. The adjuvant composition of Claim 45, wherein said composition further comprises a peptide derived from *Mycobacterium tuberculosis*.

53. A method for increasing effector cell immunity in an animal, said method comprising administering to an animal an effective amount of an adjuvant composition comprising an immunogen and an isolated nucleic acid molecule encoding a superantigen.

54. The method of Claim 53, wherein said adjuvant composition further comprises a nucleic acid molecule encoding a protein selected from the group consisting of a cytokine, a chemokine and mixtures thereof.

55. The method of Claim 53, wherein said adjuvant composition further comprises a pharmaceutically acceptable carrier.

56. The method of Claim 53, wherein said step of administering comprises injecting said adjuvant composition by a route selected from the group consisting of intravenous, intraperitoneally, intramuscularly, intraarterially and directly into a specific tissue site.

57. The method of Claim 53, wherein said animal is a mammal.

58. The method of Claim 53, wherein said animal is selected from the from the group consisting of humans, horses, dogs, cats and cattle.

59. A method to treat an animal with cancer, said method comprising administering to an animal an effective amount of a therapeutic composition comprising: (a) an isolated nucleic acid molecule encoding a superantigen; and (b) an isolated nucleic acid molecule selected from the

group consisting of a nucleic acid molecule encoding a cytokine, a nucleic acid molecule encoding a chemokine, and mixtures thereof, wherein said nucleic acid molecules are operatively linked to one or more transcription control sequences.

60. The method of Claim 59, wherein said therapeutic composition further comprises a pharmaceutically acceptable carrier selected from the group consisting of an aqueous physiologically balanced solution, an artificial lipid-containing substrate, a natural lipid-containing substrate, an oil, an ester, a glycol, a virus and a metal particle.

61. The method of Claim 59, wherein said pharmaceutically acceptable carrier is selected from the group consisting of liposomes, micelles, cells and cellular membranes.

62. The method of Claim 59, wherein said pharmaceutically acceptable carrier comprises a liposome.

63. The method of Claim 59, wherein said pharmaceutically acceptable carrier comprises a liposome that includes a compound capable of specifically targeting said liposome to a tumor cell.

64. The method of Claim 63, wherein said compound is a tumor cell ligand.

65. The method of Claim 59, wherein said therapeutic composition is targeted to the site of a cancer in said animal by administering said therapeutic composition locally within the area of a cancer cell.

66. The method of Claim 59, wherein said cancer is selected from the group consisting of melanomas, squamous cell carcinoma, breast cancers, head and neck carcinomas, thyroid carcinomas, soft tissue sarcomas, bone sarcomas, testicular cancers, prostatic cancers, ovarian cancers, bladder cancers, skin cancers, brain cancers, angiosarcomas, hemangiosarcomas, mast cell tumors, primary hepatic cancers, lung cancers, pancreatic cancers, gastrointestinal cancers, renal cell carcinomas, hematopoietic neoplasias, leukemias and lymphomas.

67. A method to treat an animal with cancer, said method comprising introducing into a tumor cell *in vivo* an effective amount of a therapeutic composition comprising an isolated nucleic acid molecule encoding a superantigen, wherein said nucleic acid molecules are operatively linked to one or more transcription control sequences.

68. The method of Claim 67, wherein said isolated nucleic acid molecule encoding a superantigen comprises a recombinant molecule selected from the group consisting of PCR_3-SEB, PCR_3-SEA, PCR_3-SEB.S, PCR_3-SEA.S and PCR_3-TSST.

69. The method of Claim 67, wherein said therapeutic composition further comprises a pharmaceutically acceptable carrier comprising a liposome.

70. The method of Claim 67, wherein said therapeutic composition further comprises a pharmaceutically acceptable carrier comprising a liposome that includes a compound

capable of specifically targeting said liposome to a tumor cell.

71. The method of Claim 70, wherein said compound is a tumor cell ligand.

72. The method of Claim 67, wherein said therapeutic composition is targeted to the site of a cancer in said animal by administering said therapeutic composition locally within the area of a cancer cell.

73. The method of Claim 67, wherein said therapeutic composition is administered to said animal at a site comprising a lymph node.

74. A method to treat an animal with cancer, said method comprising introducing into a non-tumor cell an effective amount of a therapeutic composition comprising an isolated nucleic acid molecule encoding a superantigen, wherein said nucleic acid molecules are operatively linked to one or more transcription control sequences.

75. The method of Claim 74, wherein said step of administration is performed *in vivo*.

76. The method of Claim 74, wherein said isolated nucleic acid molecule encoding a superantigen comprises a recombinant molecule selected from the group consisting of PCR_3-SEB, PCR_3-SEA, PCR_3-SEB.S, PCR_3-SEA.S and PCR_3-TSST.

77. The method of Claim 74, wherein said therapeutic composition comprises a pharmaceutically acceptable carrier comprising a liposome.

78. The method of Claim 74, wherein said therapeutic composition is targeted to the site of a cancer in said animal by administering said therapeutic composition locally within the area of a cancer cell.

79. The method of Claim 74, wherein said therapeutic composition is administered to said animal at a site comprising a lymph node.

80. A method to treat an animal with cancer, said method comprising administering to an animal an effective amount of a therapeutic composition comprising: (a) an isolated nucleic acid molecule encoding a superantigen; and (b) an isolated nucleic acid molecule encoding a cytokine, wherein said nucleic acid molecules are operatively linked to one or more transcription control sequences, and wherein said therapeutic composition is targeted to the site of a cancer in said animal to treat said cancer.

81. The method of Claim 80, wherein said superantigen is selected from the group consisting of staphylococcal enterotoxins, retroviral antigens, streptococcal antigens, mycoplasma antigens, mycobacteria antigens, viral antigens and protozoan antigens.

82. The method of Claim 80, wherein said superantigen comprises staphylococcal enterotoxins.

83. The method of Claim 80, wherein said superantigen is selected from the group consisting of SEA, SEB, SEC_1, SEC_2, SEC_3, SED, SEE and TSST.

84. The method of Claim 80, wherein said superantigen is derived from a virus selected from the group consisting of mouse mammary tumor virus, rabies virus and herpes virus.

85. The method of Claim 80, wherein said cytokine is selected from the group consisting of hematopoietic growth factors, interleukins, interferons, immunoglobulin superfamily molecules, tumor necrosis factor family molecules and chemokines.

86. The method of Claim 80, wherein said cytokine is selected from the group consisting of granulocyte macrophage colony stimulating factor, macrophage colony stimulating factor, tumor necrosis factor α, interleukin-1, interleukin-6 and interleukin-12.

87. The method of Claim 80, wherein said cytokine is selected from the group consisting of granulocyte macrophage colony stimulating factor and tumor necrosis factor α.

88. The method of Claim 80, wherein said cytokine comprises granulocyte macrophage colony stimulating factor.

89. The method of Claim 80, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences capable of being expressed in a mammalian cell.

90. The method of Claim 80, wherein said transcription control sequences are selected from the group consisting of RSV control sequences, CMV control sequences,

retroviral LTR sequences, SV-40 control sequences and β-actin control sequences.

91. The method of Claim 80, wherein said isolated nucleic acid molecule comprises a recombinant molecule selected from the group consisting of PCR_3-SEB, PCR_3-SEA, PCR_3-SEB.S, PCR_3-SEA.S, PCR_3-TSST and PCR_3-GM_3.

92. The method of Claim 80, wherein said therapeutic composition further comprises a pharmaceutically acceptable carrier selected from the group consisting of an aqueous physiologically balanced solution, an artificial lipid-containing substrate, a natural lipid-containing substrate, an oil, an ester, a glycol, a virus and a metal particle.

93. The method of Claim 92, wherein said pharmaceutically acceptable carrier is selected from the group consisting of liposomes, micelles, cells and cellular membranes.

94. The method of Claim 92, wherein said pharmaceutically acceptable carrier comprises a liposome.

95. The method of Claim 92, wherein said pharmaceutically acceptable carrier comprises a liposome that includes a compound capable of specifically targeting said liposome to a tumor cell.

96. The method of Claim 95, wherein said compound is a tumor cell ligand.

97. The method of Claim 80, wherein said therapeutic composition is targeted to the site of a cancer in said

animal by administering said therapeutic composition locally within the area of a cancer cell.

98. The method of Claim 80, wherein said cancer is selected from the group consisting of melanomas, squamous cell carcinoma, breast cancers, head and neck carcinomas, thyroid carcinomas, soft tissue sarcomas, bone sarcomas, testicular cancers, prostatic cancers, ovarian cancers, bladder cancers, skin cancers, brain cancers, angiosarcomas, hemangiosarcomas, mast cell tumors, primary hepatic cancers, lung cancers, pancreatic cancers, gastrointestinal cancers, renal cell carcinomas, hematopoietic neoplasias, leukemias and lymphomas.

99. The method of Claim 80, wherein said cancer is selected from the group consisting of melanomas, lung cancers, thyroid carcinomas, breast cancers, renal cell carcinomas, squamous cell carcinomas, brain tumors and skin cancers.

100. The method of Claim 80, wherein said animal is selected from the group consisting of mammals and birds.

101. The method of Claim 80, wherein said animal is selected from the from the group consisting of humans, house pets, economic produce animals and zoo animals.

102. The method of Claim 80, wherein said animal is selected from the from the group consisting of humans, dogs, cats, sheep, cattle, horses and pigs.

103. A therapeutic composition comprising an isolated nucleic acid molecule encoding a superantigen and an

isolated nucleic acid molecule encoding a cytokine, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences.

104. The composition of Claim 103, wherein said therapeutic composition further comprises a pharmaceutically acceptable carrier.

105. The composition of Claim 104, wherein said pharmaceutically acceptable carrier is selected from the group consisting of an aqueous physiologically balanced solution, an artificial lipid-containing substrate, a natural lipid-containing substrate, an oil, an ester, a glycol, a virus and metal particles.

106. The composition of Claim 104, wherein said pharmaceutically acceptable carrier comprises a delivery vehicle capable of delivering said nucleic acid molecules to a targeted site in an animal.

107. The composition of Claim 106, wherein said delivery vehicle is selected from the group consisting of liposomes, micelles, cells and cellular membranes.

108. The composition of Claim 106, wherein said delivery vehicle comprises a liposome.

109. The composition of Claim 106, wherein said delivery vehicle comprises a tumor cell ligand.

110. The composition of Claim 103, wherein said superantigen is selected from the group consisting of staphylococcal enterotoxins, retroviral antigens,

streptococcal antigens, mycoplasma antigens, mycobacteria antigens, virus antigens and protozoan antigens.

111. The composition of Claim 103, wherein said superantigen comprises staphylococcal enterotoxins.

112. The composition of Claim 103, wherein said superantigen is selected from the group consisting of SEA, SEB, SEC_1, SEC_2, SEC_3, SED, SEE and TSST.

113. The composition of Claim 103, wherein said superantigen is derived from a virus selected from the group consisting of mouse mammary tumor virus, rabies virus and herpes virus.

114. The composition of Claim 103, wherein said cytokine is selected from the group consisting of hematopoietic growth factors, interleukins, interferons, immunoglobulin superfamily molecules, tumor necrosis factor family molecules and chemokines.

115. The composition of Claim 103, wherein said cytokine is selected from the group consisting of granulocyte macrophage colony stimulating factor, macrophage colony stimulating factor, tumor necrosis factor α, interleukin-1, interleukin-6 and interleukin-12.

116. The composition of Claim 103, wherein said cytokine is granulocyte macrophage colony stimulating factor and tumor necrosis factor α.

117. The composition of Claim 103, wherein said isolated nucleic acid molecules are operatively linked to

one or more transcription control sequences capable of being expressed in a mammalian cell.

118. The composition of Claim 103, wherein said transcription control sequences are selected from the group consisting of RSV control sequences, CMV control sequences, retroviral LTR control sequences, SV-40 control sequences and β-actin control sequences.

119. The composition of Claim 103, wherein said nucleic acid molecule comprises a recombinant molecule selected from the group consisting of PCR_3-SEB, PCR_3-SEA, PCR_3-SEB.S, PCR_3-SEA.S, PCR_3-TSST and PCR_3-GM_3.

120. The composition of Claim 103, wherein said therapeutic composition is useful for treating a cancer selected from the group consisting of melanomas, squamous cell carcinoma, breast cancers, head and neck carcinomas, thyroid carcinomas, soft tissue sarcomas, bone sarcomas, testicular cancers, prostatic cancers, ovarian cancers, bladder cancers, skin cancers, brain cancers, angiosarcomas, hemangiosarcomas, mast cell tumors, primary hepatic cancers, lung cancers, pancreatic cancers, gastrointestinal cancers, renal cell carcinomas, hematopoietic neoplasias, leukemias and lymphomas.

121. The composition of Claim 103, wherein said therapeutic composition is useful for treating a cancer selected from the group consisting of melanomas, lung cancers, thyroid carcinomas, breast cancers, renal cell

carcinomas, squamous cell carcinomas, brain tumors and skin cancers.

122. A recombinant molecule comprising an isolated nucleic acid molecule encoding a superantigen and an isolated nucleic acid molecule encoding a cytokine, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences.

123. The molecule of Claim 122, wherein said recombinant molecule is capable being expressed in a mammalian cell.

124. The molecule of Claim 122, wherein said recombinant molecule contains a transcription control sequence selected from the group consisting of RSV control sequences, CMV control sequences, retroviral LTR control sequences, SV-40 control sequences and β-actin control sequences.

125. The molecule of Claim 122, wherein said superantigen-encoding nucleic acid molecule encodes a toxin selected from the group consisting of staphylococcal enterotoxins, retroviral antigens, streptococcal antigens, mycoplasma antigens, mycobacteria antigens, virus antigens and protozoan antigens.

126. The molecule of Claim 122, wherein said superantigen-encoding nucleic acid molecule encodes a toxin selected from the group consisting of a SEA, SEB, SEC_1, SEC_2, SEC_3, SED, SEE and TSST gene.

127. The molecule of Claim 122, wherein said superantigen-encoding nucleic acid molecule lacks a bacterial leader sequence.

128. The molecule of Claim 122, wherein said recombinant molecule is selected from the group consisting of PCR_3-SEB, PCR_3-SEA, PCR_3-SEB.S, PCR_3-SEA.S, PCR_3-TSST and PCR_3-GM_3.

129. A therapeutic composition comprising a delivery vehicle carrying an isolated nucleic acid molecule encoding a superantigen and an isolated nucleic acid molecule encoding a cytokine, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences.

130. The composition of Claim 129, wherein said delivery vehicle is selected from the group consisting of a liposome, a micelle, a cell and a cellular membrane.

131. The composition of Claim 129, wherein said delivery vehicle comprises a liposome.

132. The composition of Claim 129, wherein said delivery vehicle comprises DOTMA and DOPE.

133. The composition of Claim 132, wherein said compound is a tumor cell ligand.

134. A method for increasing effector cell immunity in an animal, said method comprising administering to an animal an effective amount of a therapeutic composition comprising:

a) an isolated nucleic acid molecule encoding a superantigen; and

b) an isolated nucleic acid molecule encoding a cytokine,

wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences, and wherein said therapeutic composition is targeted to a site in said animal that contains an abnormal cell.

135. The method of Claim 134, wherein said abnormal cell is selected from the group consisting of a cancer cell, a cell infected with an infectious agent and a non-cancerous cell having abnormal proliferative growth.

136. The method of Claim 134, wherein said abnormal cell is a cancer cell.

137. The method of Claim 134, wherein said site is a tumor.

138. A method to suppress T cell activity in an animal, said method comprising administering to an animal an effective amount of a therapeutic composition comprising:

a) a naked isolated nucleic acid molecule encoding a superantigen; and

b) a pharmaceutically acceptable carrier,

wherein said isolated nucleic acid molecule is operatively linked to a traynscription control sequence, and wherein said therapeutic composition is targeted to a

site in said animal that contains excessive T cell activity.

139. The method of Claim 138, wherein said composition is capable of suppressing said T cell activity for at least about 6 weeks in said animal.

140. The method of Claim 138, wherein said composition is capable of suppressing said T cell activity for at least about 8 weeks in said animal.

141. The method of Claim 138, wherein said composition is capable of suppressing said T cell activity for about 10 weeks in said animal.

142. The method of Claim 138, wherein said carrier comprises an aqueous physiologically balanced solution.

3 Different Bacterial Superantigens (SEB, SEA, TSST) Can Be Expressed in Eukaryotic Cells

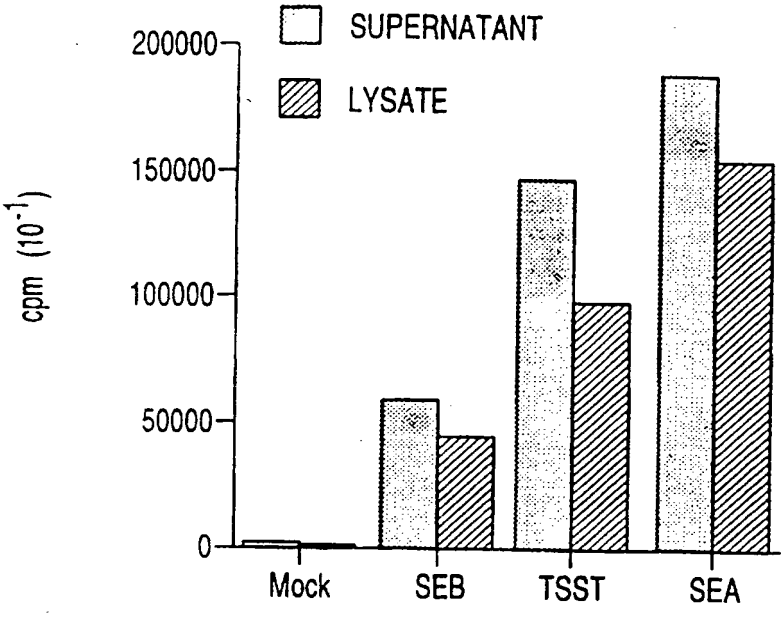

Fig. 1

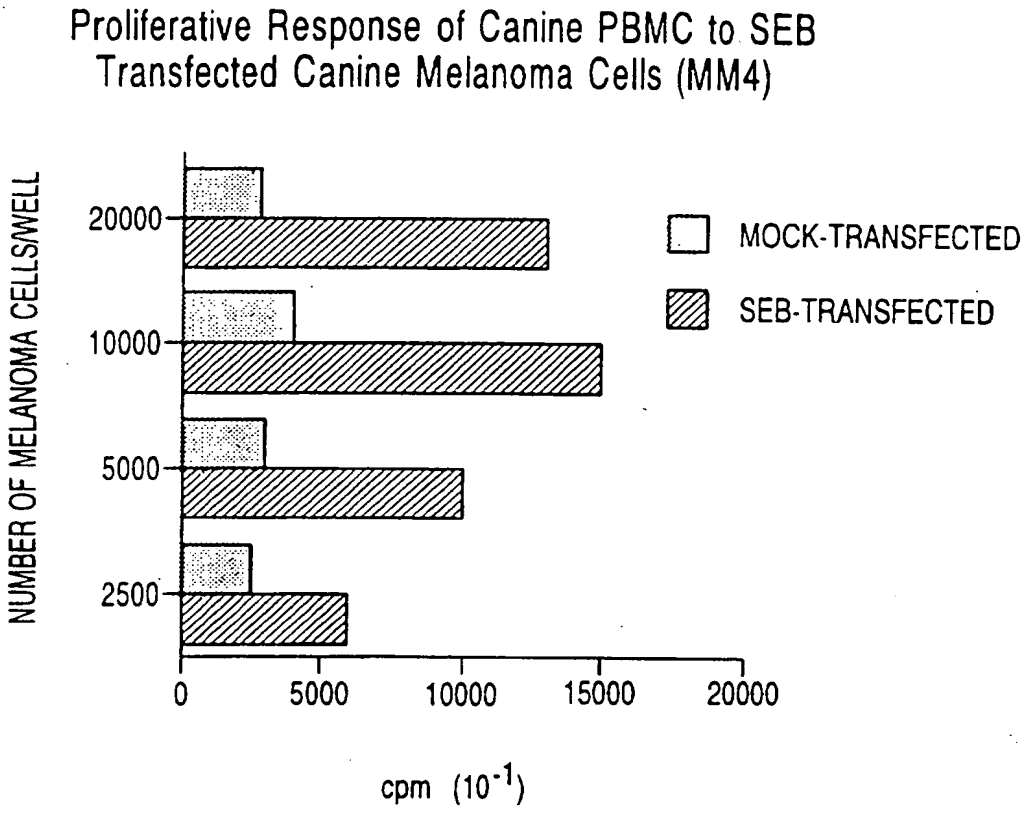
Proliferative Response of Canine PBMC to SEB Transfected Canine Melanoma Cells (MM4)
NUMBER OF MELANOMA CELLS/WELL
20000
10000
5000
2500
0
5000
10000
15000
20000
cpm (10^{-1})
MOCK-TRANSFECTED
SEB-TRANSFECTED

Fig. 2

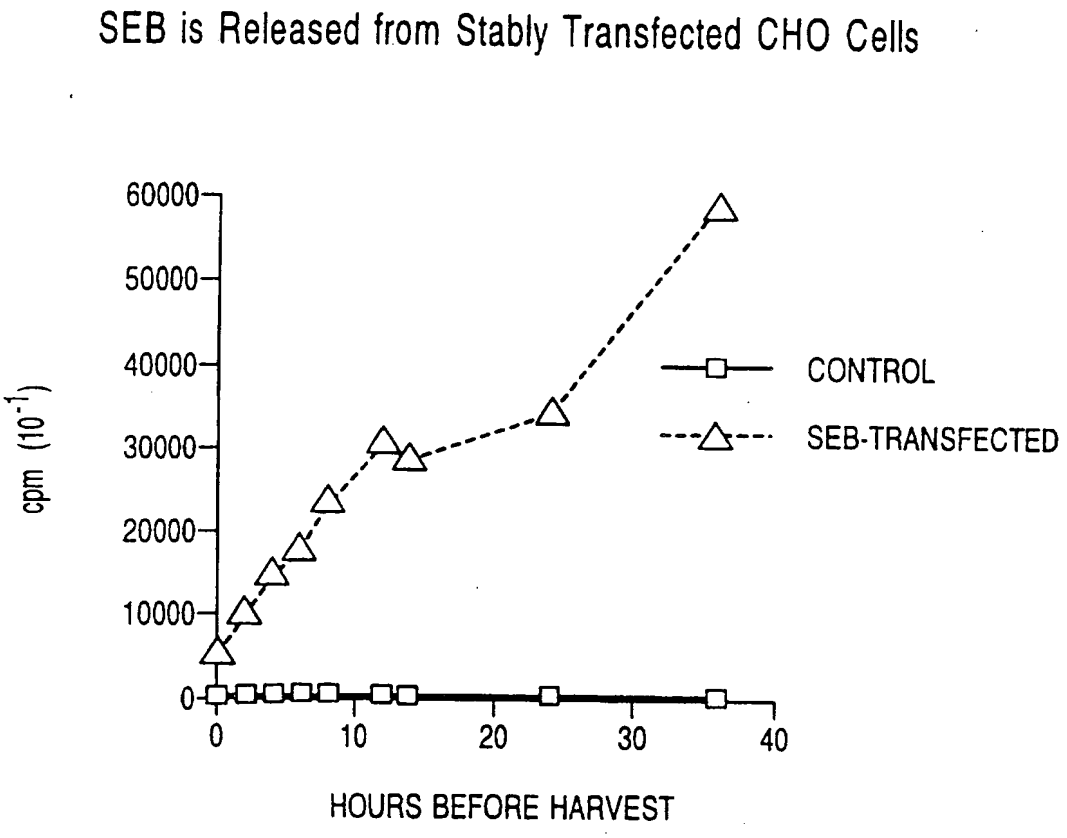
SEB is Released from Stably Transfected CHO Cells
60000
50000
40000
30000
20000
10000
0
cpm (10^{-1})
0
10
20
30
40
HOURS BEFORE HARVEST
CONTROL
SEB-TRANSFECTED

Fig. 3A

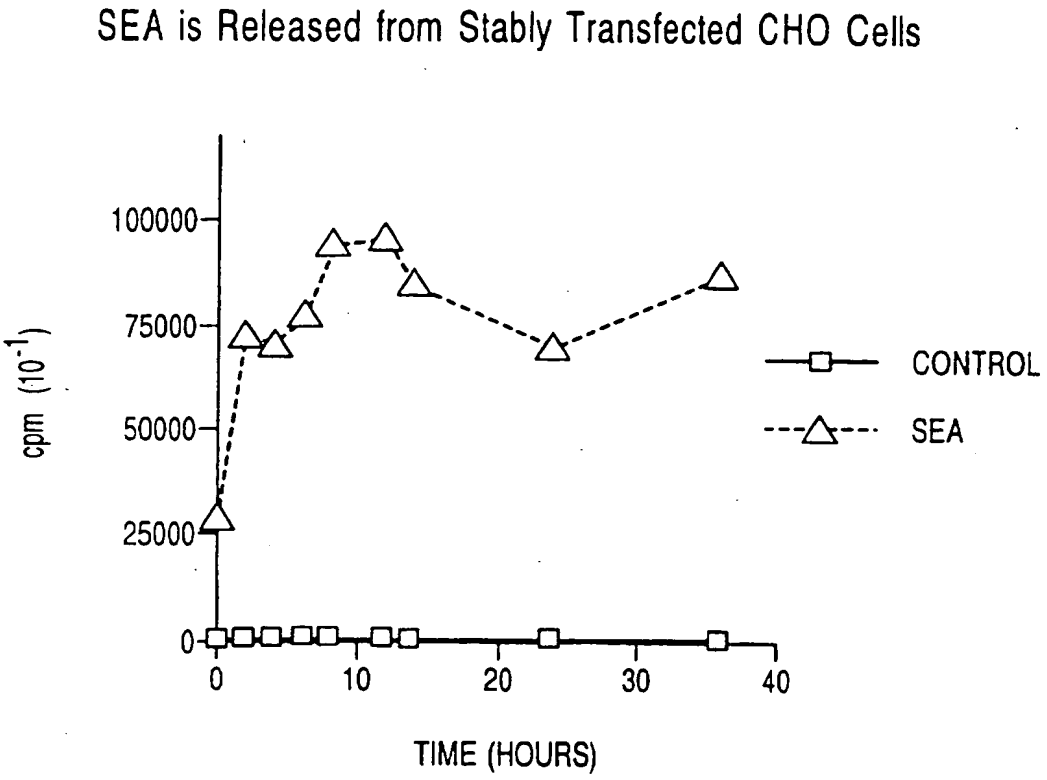
SEA is Released from Stably Transfected CHO Cells
100000
75000
50000
25000
0
cpm (10^{-1})
0
10
20
30
40
TIME (HOURS)
CONTROL
SEA

Fig. 3B

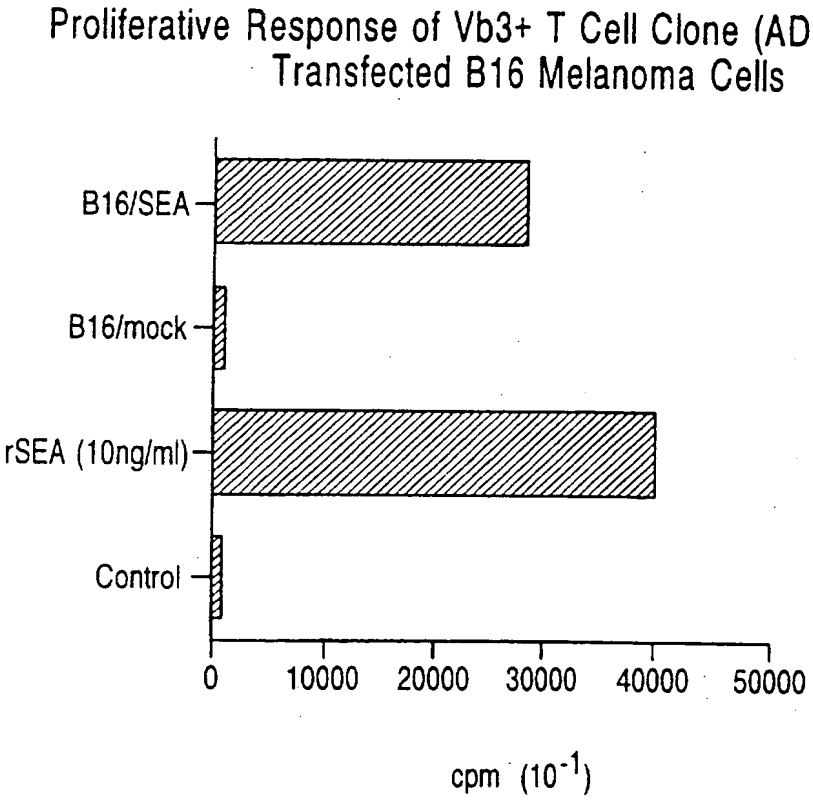
Proliferative Response of Vb3+ T Cell Clone (AD10) to SEA Transfected B16 Melanoma Cells
B16/SEA
B16/mock
rSEA (10ng/ml)
Control
0
10000
20000
30000
40000
50000
cpm (10-1)

Fig. 4

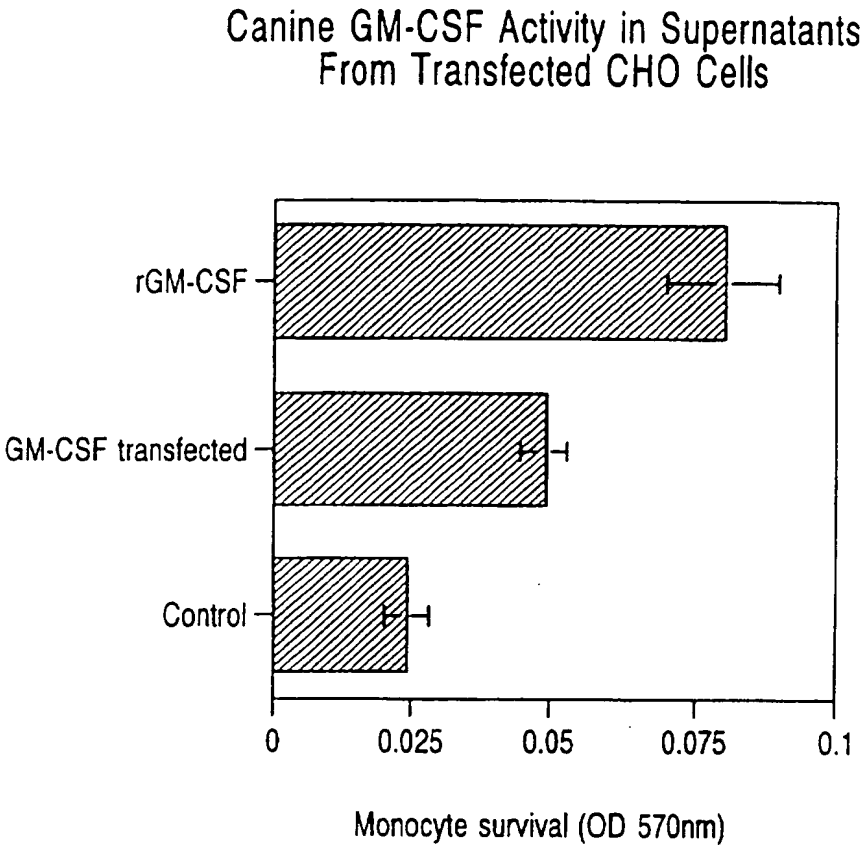
Canine GM-CSF Activity in Supernatants
From Transfected CHO Cells
rGM-CSF
GM-CSF transfected
Control
0
0.025
0.05
0.075
0.1
Monocyte survival (OD 570nm)

Fig. 5

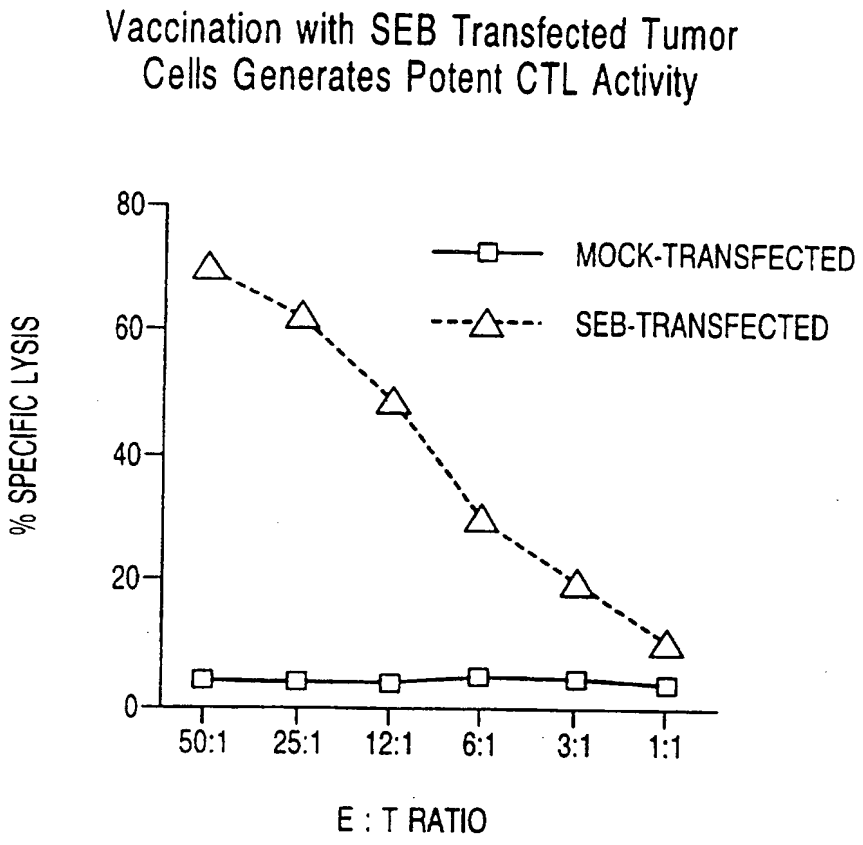
Vaccination with SEB Transfected Tumor Cells Generates Potent CTL Activity
% SPECIFIC LYSIS
80
60
40
20
0
MOCK-TRANSFECTED
SEB-TRANSFECTED
50:1
25:1
12:1
6:1
3:1
1:1
E : T RATIO

Fig. 6A

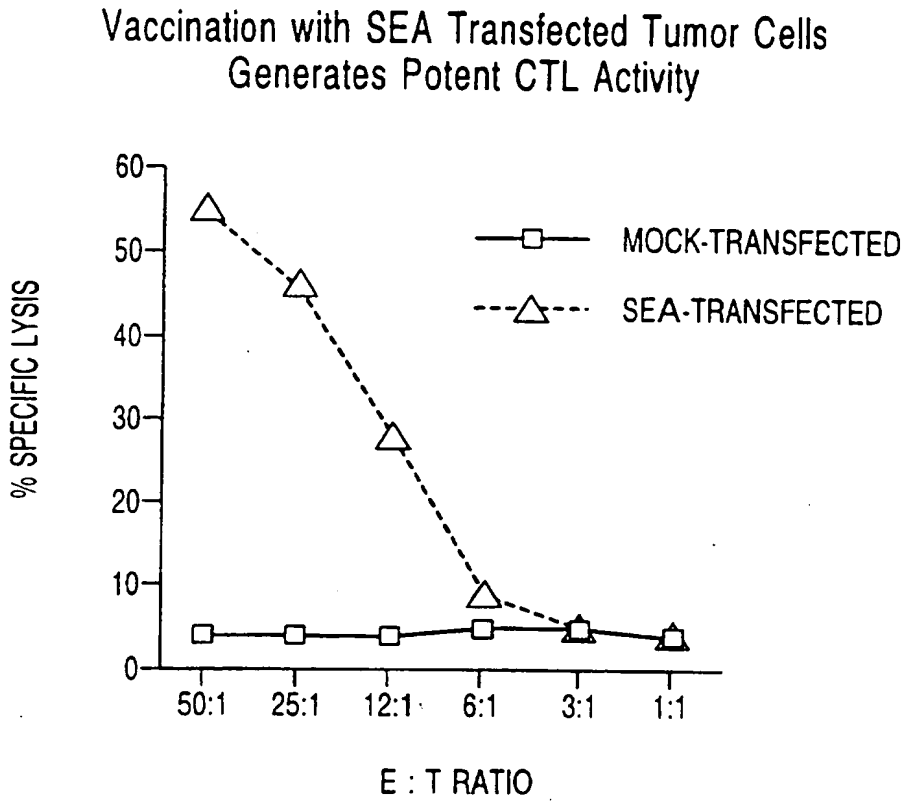
Vaccination with SEA Transfected Tumor Cells
Generates Potent CTL Activity
% SPECIFIC LYSIS
60
50
40
30
20
10
0
MOCK-TRANSFECTED
SEA-TRANSFECTED
50:1
25:1
12:1
6:1
3:1
1:1
E : T RATIO

Fig. 6B

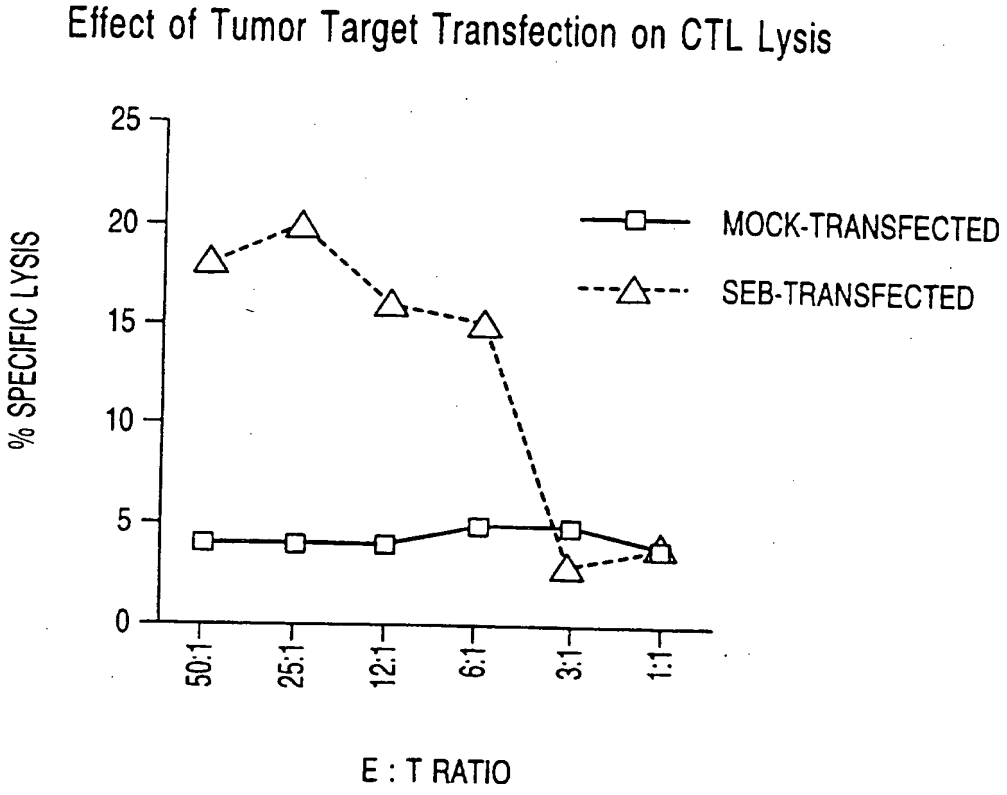
Effect of Tumor Target Transfection on CTL Lysis
% SPECIFIC LYSIS
25
20
15
10
5
0
50:1
25:1
12:1
6:1
3:1
1:1
E : T RATIO
MOCK-TRANSFECTED
SEB-TRANSFECTED

Fig. 7

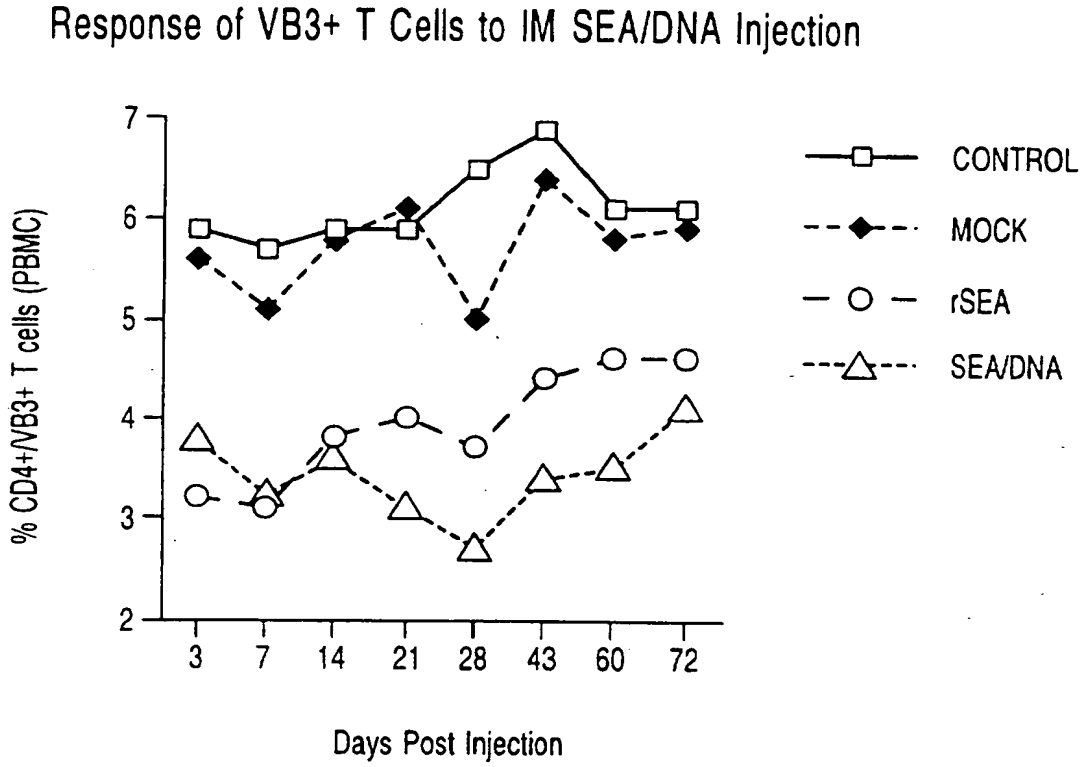
Response of VB3+ T Cells to IM SEA/DNA Injection
% CD4+/VB3+ T cells (PBMC)
7
6
5
4
3
2
3
7
14
21
28
43
60
72
Days Post Injection
CONTROL
MOCK
rSEA
SEA/DNA

Fig. 8

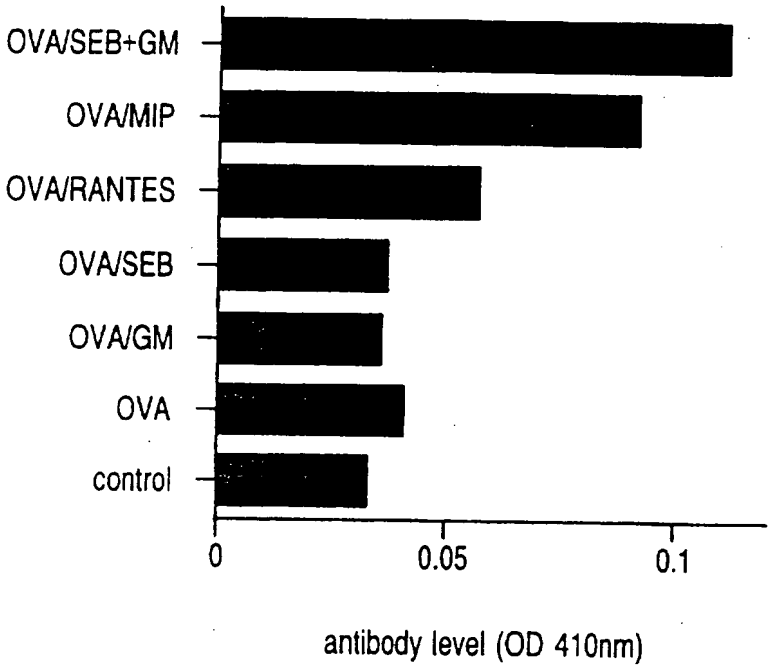
Antibody Response to Single OVA DNA Injection (day 20)
OVA/SEB+GM
OVA/MIP
OVA/RANTES
OVA/SEB
OVA/GM
OVA
control
0
0.05
0.1
antibody level (OD 410nm)

Fig. 9

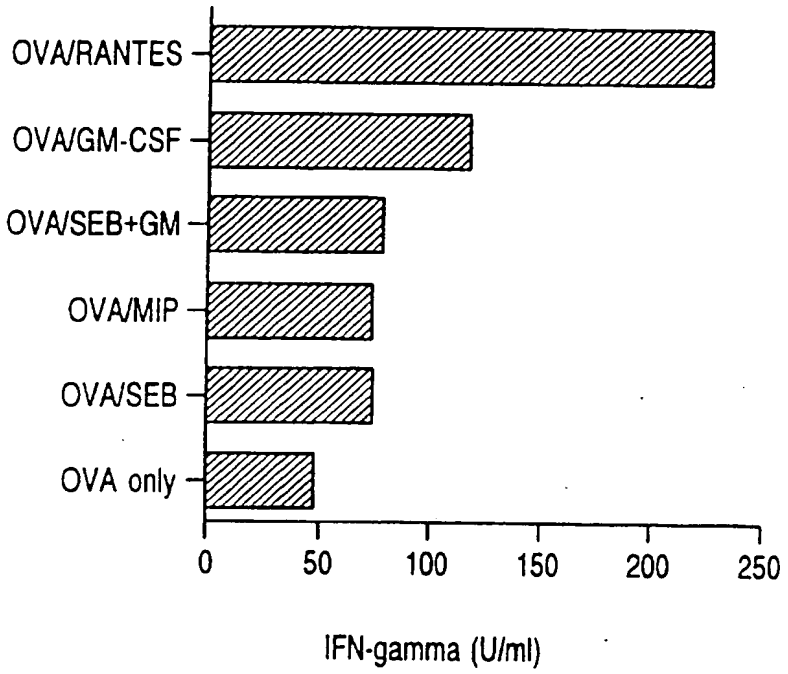
Interferon Gamma Release after Stimulation with EG7-OVA
OVA/RANTES
OVA/GM-CSF
OVA/SEB+GM
OVA/MIP
OVA/SEB
OVA only
0
50
100
150
200
250
IFN-gamma (U/ml)

Fig. 10

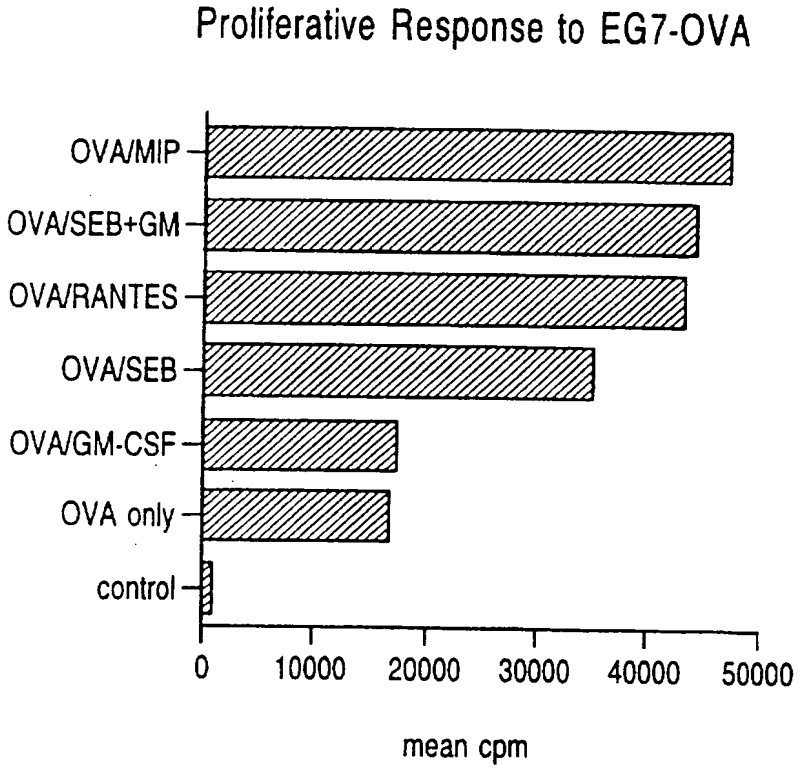
Proliferative Response to EG7-OVA
OVA/MIP
OVA/SEB+GM
OVA/RANTES
OVA/SEB
OVA/GM-CSF
OVA only
control
0
10000
20000
30000
40000
50000
mean cpm

Fig. 11

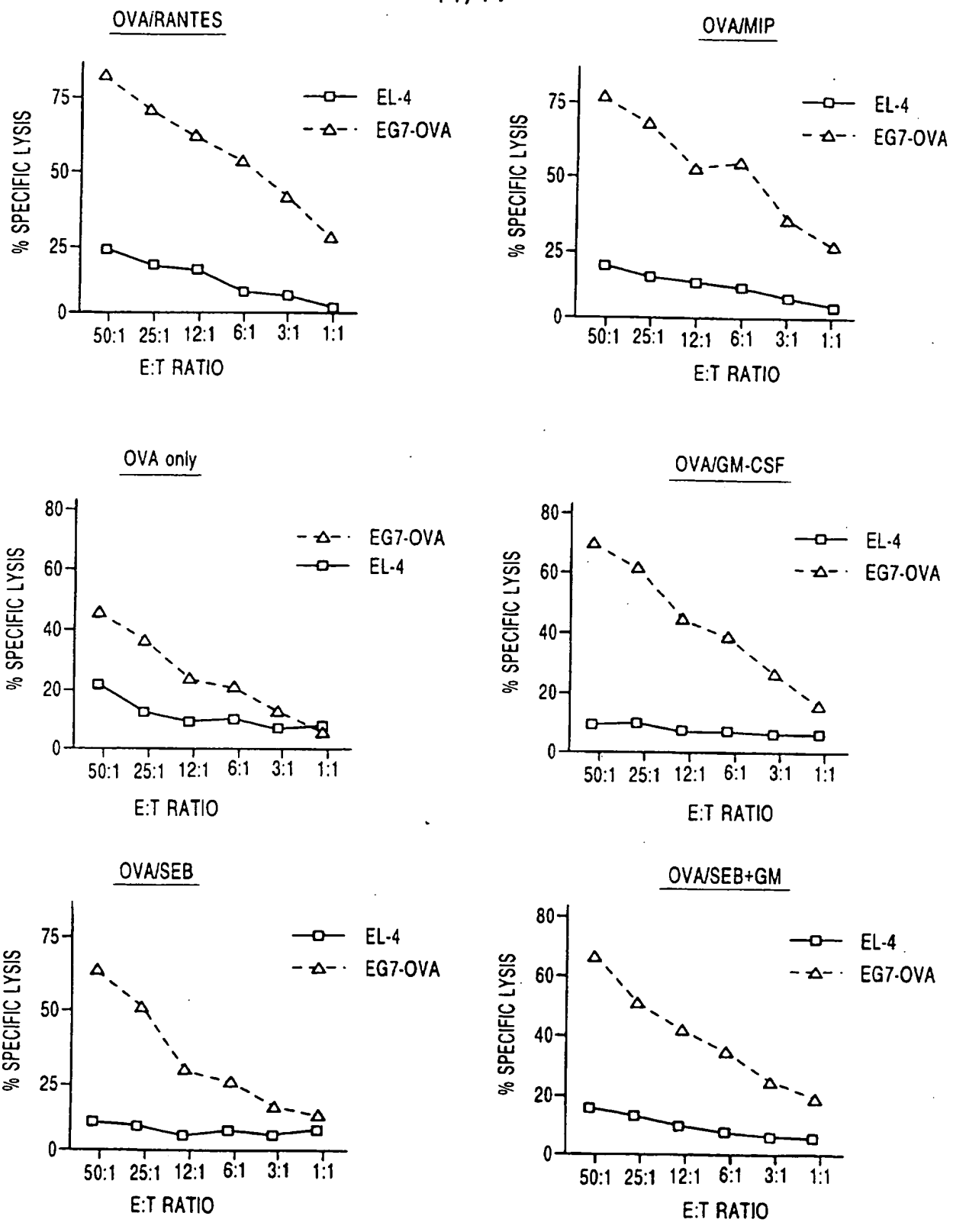
OVA/RANTES
% SPECIFIC LYSIS
75
50
25
0
EL-4
EG7-OVA
50:1 25:1 12:1 6:1 3:1 1:1
E:T RATIO
OVA/MIP
% SPECIFIC LYSIS
75
50
25
0
EL-4
EG7-OVA
50:1 25:1 12:1 6:1 3:1 1:1
E:T RATIO
OVA only
% SPECIFIC LYSIS
80
60
40
20
0
EG7-OVA
EL-4
50:1 25:1 12:1 6:1 3:1 1:1
E:T RATIO
OVA/GM-CSF
% SPECIFIC LYSIS
80
60
40
20
0
EL-4
EG7-OVA
50:1 25:1 12:1 6:1 3:1 1:1
E:T RATIO
OVA/SEB
% SPECIFIC LYSIS
75
50
25
0
EL-4
EG7-OVA
50:1 25:1 12:1 6:1 3:1 1:1
E:T RATIO
OVA/SEB+GM
% SPECIFIC LYSIS
80
60
40
20
0
EL-4
EG7-OVA
50:1 25:1 12:1 6:1 3:1 1:1
E:T RATIO

Fig. 12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/07432

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :A61K 48/00
US CL :514/44
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
APS, MEDLINE, EMBASE, BIOSIS, CAPLUS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 95/00178 A1 (BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM) 05 January 1995, see entire document.	1-142
Y	DONNELLY et al. Protective Efficacy of Intramuscular Immunization with Naked DNA. Ann. N.Y. Acad. Sci. 1995, Vol.772, pages 40-46, see entire document.	1-142
Y	BLACKMAN et al. In Vivo Effects of Superantigens. Life Sciences. 1995, Vol.57, No.19, pages 1717-1735, see entire document.	1-142
Y	MIETHKE et al. Superantigen Mediated Shock: A Cytokine Release Syndrome. Immunobiol. 1993, Vol.189, pages 270-284, see entire document.	1-142

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
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"E" earlier document published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
25 JULY 1996

Date of mailing of the international search report
23 AUG 1996

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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/07432

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MIETHKE et al. Superantigens: The Paradox of T-Cell Activation versus Inactivation. Int. Arch. Allergy Immunol. 1995, Vol.106, pages 3-7, see entire document.	1-142
Y	LIU et al. Overview of DNA Vaccines. Ann. N.Y. Acad. Sci. 1995, Vol.772, pages 15-20, see entire document.	1-142